Polycystic Kidney Disease Outcomes Consortium

Qualification of Total Kidney Volume as a Prognostic Biomarker for use in Clinical Trials Evaluating Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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ACEI	Angiotensin-converting-enzyme inhibitor
ACR	Albumin to creatinine ratio
ADPKD	Autosomal Dominant Polycystic Kidney Disease
AIC	Akaike Information Criterion
AKI	Acute kidney injury
BM	Biomarker
BMI	Body Mass Index
BP	Blood pressure
BUN	Blood urea nitrogen
BQRT	Biomarker Qualification Review Team
CDISC	Clinical Data Interchange Standards Consortium
CF	Cystic fibrosis
CI	Confidence intervals
CKD	Chronic kidney disease
CRISP	Consortium of Radiological Imaging Studies of Polycystic Kidney Disease (including CRISP I and CRISP II)
C-Path	Critical Path Institute
CrCl	Creatinine clearance
CT	Computed Tomography
CWRES	Conditional weighted residuals
DV	Dependent variable
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
ESRD	End-stage renal disease
FDA	Food and Drug Administration
FN	False negative
FOCE	First-order conditional estimation
FP	False positive
GCRC	General Clinical Research Center
GFR	Glomerular filtration rate
GINA	Genetic Information Nondiscrimination Act
GM	Geometric mean
HA	Height-adjusted
IPRED	Individual predicted TKV values
IRB	Institutional Review Board
KDOQI-CKD	Kidney Disease Outcomes Quality Initiative-Chronic Kidney Disease
KW	Kidney weight
LOESS	Locally weighted scatter plot smoothing
LOI	Letter of Intent
MAD	Multiple ascending dose
MOF	Minimum value of objective function

$\triangle$MOF	Difference between the MOF values of a reference model and of a tested model
MR	Magnetic Resonance
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
NFD	Nephrogenic Fibrosing Dermopathy
NSF	Nephrogenic Systemic Fibrosis
NLME	Nonlinear mixed-effect
NSAIDs	Non-steroidal anti-inflammatory drugs
OBJ	Objective function
PCC	Participating Clinical Center
PKDOC	Polycystic Kidney Disease Outcomes Consortium
PKD1	Genetic form of ADPKD caused by mutations in PKD-1 gene (85-90% of cases)
PKD2	Genetic form of ADPKD caused by mutations in PKD-2 gene (10-15% of cases)
PRED	Population predicted values
QC	Quality control
QQ	Quantile-quantile plot
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomized Controlled Trial
RICA	Ruptured intracranial aneurysm
RIFLE	Risk Injury Failure Loss End-stage renal disease
ROC	Receiver operator characteristics
SAD	Single ascending dose
SAWP	Scientific Advice Working Party
sCr	Serum creatinine
SOC	Standard of care
SOPs	Standard operating procedures
SDTM	Study Data Tabulation Model
TCV	Total cyst volume
TKV	Total kidney volume
TN	True negative
TP	True positive
ULN	Upper limit of normal
US	Ultrasonography
USRDS	United States Renal Data System
VXDS	Voluntary Exploratory Data Submission

2   Executive Summary

This Briefing Document is submitted on behalf of the Polycystic Kidney Disease Outcomes Consortium (PKDOC) to the Biomarkers Qualification Review Team (BQRT) at the U.S. Food and Drug Administration (FDA) and the Scientific Advice Working Party (SAWP) at the European Medicines Agency (EMA) for the qualification of Total Kidney Volume (TKV) as a prognostic biomarker for the following Context of Use:

- **General Area**: Clinical trial enrichment in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

- **Target Population for Use**: Patients with ADPKD

- **Stage of Drug Development for Use**: All clinical stages of ADPKD drug development, including proof of concept, dose-ranging, and confirmatory clinical trials.

- **Intended Application:** Baseline TKV can be applied as a prognostic biomarker that, in combination with patient age and baseline estimated Glomerular Filtration Rate (eGFR), can be used to help identify those ADPKD patients who are at the greatest risk for a substantial decline in renal function defined as (1) 30% worsening of eGFR, (2) 57% worsening of eGFR (equivalent to doubling of serum creatinine), or (3) End-Stage Renal Disease (ESRD, defined as dialysis or transplant). This biomarker will be used as an inclusion criterion in clinical trials to identify patients likely to show a clinically relevant decline in kidney function during the duration of the trial. Data are provided showing the calculated risk of each of these outcomes of declining renal function depending on age, total kidney volume, and baseline eGFR. Tables will be used by clinical trial researchers to determine the inclusion criteria to help select patients who are likely to reach the clinical endpoint of interest within a timeframe practical for the trial. These criteria include the optimum age, TKV, and eGFR for selecting subjects to be enrolled in the clinical trial.

   Kidney volume can be measured by Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scan, or ultrasound (US) imaging, and the volume calculated by a standard methodology, such as an ellipsoid volume equation (for ultrasound), or by quantitative stereology or boundary tracing (for CT/MRI).

A formal Letter of Intent (LOI) was submitted to the FDA on January 3rd, 2012. The FDA responded on March 6th, 2012 with an acceptance of this LOI. Included in the acceptance communication from FDA were comments and suggestions for the PKDOC to consider in their preparation of the Briefing Document. That initial Briefing Document was submitted to the FDA on September 24th, 2012, and preliminary comments from the Biomarker Qualification Review Team (BQRT) were received on November 2nd, 2012 and in follow-up conversations on November 9 and December 12, 2012. A revised Briefing Package was submitted to the FDA on April 30, 2013, and on June 28, 2013, the PKDOC met with the FDA BQRT to discuss comments and questions. Several follow-up sessions to discuss questions and requests were held in July through September, and on September 27th, 2013, the FDA approved submission of the final Briefing Book with a list of questions that should be addressed.

A formal Letter of Intent was submitted to the EMA on April 11th, 2013, followed by submission of the initial EMA Briefing Package on April 30, 2013. In response to a List of Issues provided by the EMA on

the Briefing Book, a face-to-face meeting was held in London on July 9, 2013. Following questions and responses that were addressed via email during the next several months, the EMA indicated that all remaining questions could be addressed in the submission of the final Briefing Package. See **Table 2** for a summary of all regulatory interactions.

The PKDOC is a collaboration between the Polycystic Kidney Disease (PKD) Foundation, the FDA, Critical Path Institute (C-Path), the Clinical Data Interchange Standards Consortium (CDISC), clinicians and scientists who are considered the world's leading experts in the field of ADPKD, members of the pharmaceutical industry, and patients (through the PKD Foundation). The PKD Foundation provides funding for this work. The project began initially as collaboration between the PKD Foundation and the FDA in 2007 as an effort to facilitate clinical trial development for ADPKD therapies through the qualification of TKV as a measure of disease progression.

ADPKD is the most common hereditary kidney disease. Currently there are no approved therapies to prevent, cure, stop, or even slow down the rate of disease progression in patients with ADPKD. Tremendous scientific progress has been made in understanding the mechanism of disease and pathophysiological processes underlying ADPKD. This has resulted in several potential drug therapy targets, some of which have shown great promise in animal studies. However, sponsors are currently reluctant to invest in the development of these potentially promising compounds in the absence of a clear, viable, and acceptable regulatory path with respect to clinical trial design and endpoints. The qualification of an appropriate biomarker to be used in drug development decision making will represent a significant, innovative step forward to establishing the commitment of health authorities, clinicians, and patients to address the unmet needs for this debilitating condition, thereby encouraging researchers and the pharmaceutical industry to develop promising new therapies for these patients.

The PKDOC has identified TKV as an imaging biomarker that is most promising and relevant for tracking and predicting the natural history of ADPKD. There is evidence in the literature from both animal and human studies to support TKV as a prognostic endpoint for use in clinical trials for ADPKD. However, the data currently available are in the form of anecdotal reports, or clinical studies with small number of patients and followed for limited periods of time. In discussions with the FDA, PKDOC has developed the first-ever CDISC data standard for ADPKD to allow for the mapping and pooling of available data into a common dataset that has enabled the development of quantitative modeling tools for use in this regulatory qualification submission of TKV to the FDA and EMA. This common dataset is one of the largest ever datasets of ADPKD patients, with a total of 2355 patients who have at least one measurement of TKV. Of these, a subset consisting of 1182 patients have at least two measurements of TKV taken at least six months apart. This rich and robust dataset has allowed the PKDOC to develop a predictive model linking baseline TKV (in combination with age and baseline eGFR) to specific clinical outcomes of ADPKD, supporting the regulatory qualification of this biomarker that can be applied to enrich clinical trial populations with patients most likely to demonstrate a response to, and benefit from, therapeutic interventions.

3   Background

3.1   History

ADPKD is the most common hereditary kidney disease with a phenotypic prevalence of 1:400 – 1:1000 individuals when including identification by autopsy {Iglesias 1983, Torres 2009, Wilson 2004}. Cysts develop in the kidneys of the human fetus and continue forming post-partum {Grantham 2011b, Grantham 2012}. Kidney cysts are the first recognizable features of ADPKD in humans and they continue to expand throughout life. Renal cysts are responsible for all renal manifestations of ADPKD. The development and growth of cysts over time causes increased kidney size and compression of normal renal architecture and vasculature leading to pericystic and interstitial fibrosis and kidney failure. Many, but not all, patients will suffer from increasing morbidity due to their enlarging kidneys, including severe pain, increasing abdominal girth, hypertension, gross hematuria, nephrolithiasis, urinary tract infections, cyst hemorrhage, and kidney infection. Progressive kidney dysfunction develops over decades in up to half of those diagnosed. However, some patients (especially those with PKD2) progress more slowly and die of other causes before a diagnosis can be prompted by symptoms. For those who progress to end-stage renal disease (ESRD), it has been shown that half will develop ESRD by age 53 years; however ESRD is rare below age 30 years {Hateboer 1999}. Recent data from the United States Renal Data System demonstrates that the age of ESRD in the ADPKD population in the United States has not significantly changed since 1991, remaining near 55-56 years of age {personal communication, 3/2013, Eric Weinhandl, United States Renal Data System}. See Figure 1.

Figure 1: Age of ESRD in individuals with ADPKD (from USRDS)
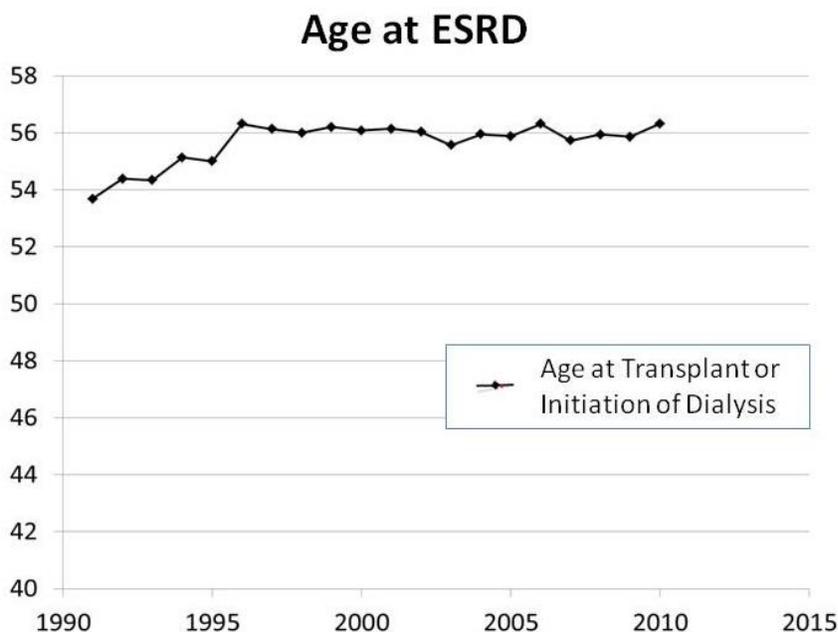
For comparison purposes and as an indicator of the generalizability of the US data above, Figure 2 provides similar data for nine European countries {Spithoven 2014; Used with permission}.

Figure 2: Age of ESRD (Renal Replacement Therapy) in individuals with ADPKD (from Europe)
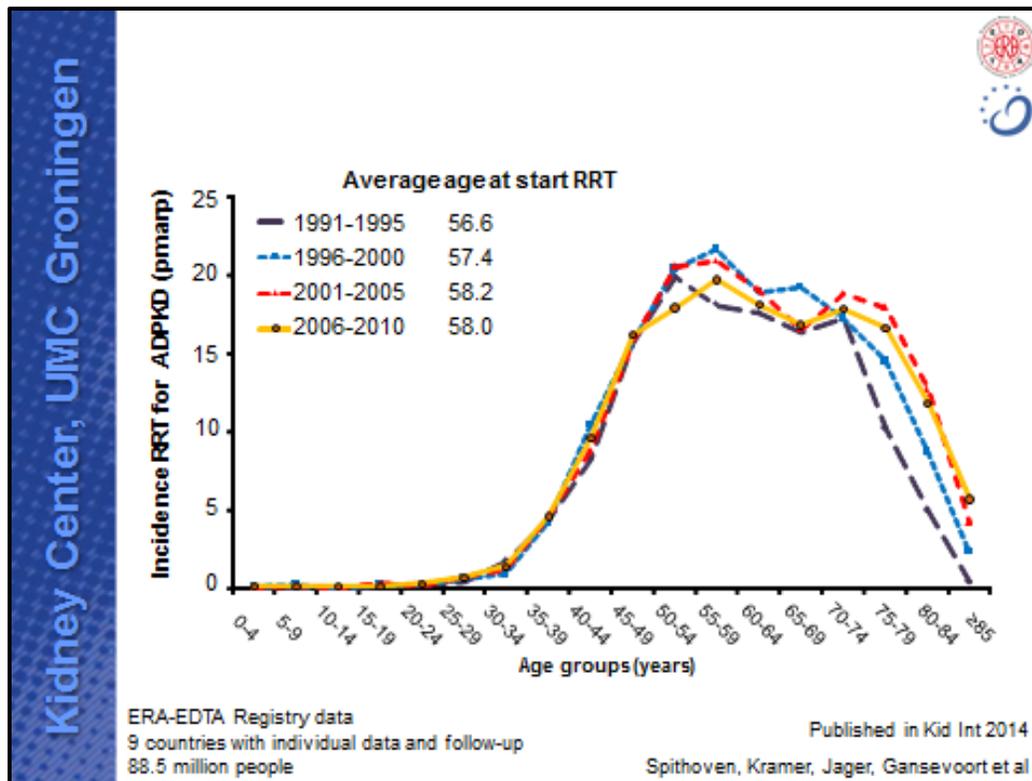
In addition, ADPKD is the fourth-leading cause of ESRD in adults, accounting for approximately 8% of the dialysis population and leads to significant morbidity.

There is no specific or targeted regulatory-approved therapy for ADPKD. Current practice focuses on strict blood pressure (BP) control, the use of statins to reduce the associated cardiac mortality, and treatment of specific complications such as pain, infection, and renal stones. In some cases, nephrectomy is the only option for intractable pain. Ultimately, for those patients who progress to ESRD, the options are limited to dialysis or renal transplantation {Takiar 2010}.

The clinical course of ADPKD is marked by a decades-long period of stable kidney function, as measured by glomerular filtration rate (GFR), despite the relentless expansion of total kidney volume (TKV) due to growth of cysts (Figure 3).

Figure 3: Increase in kidney size and change in kidney function with age
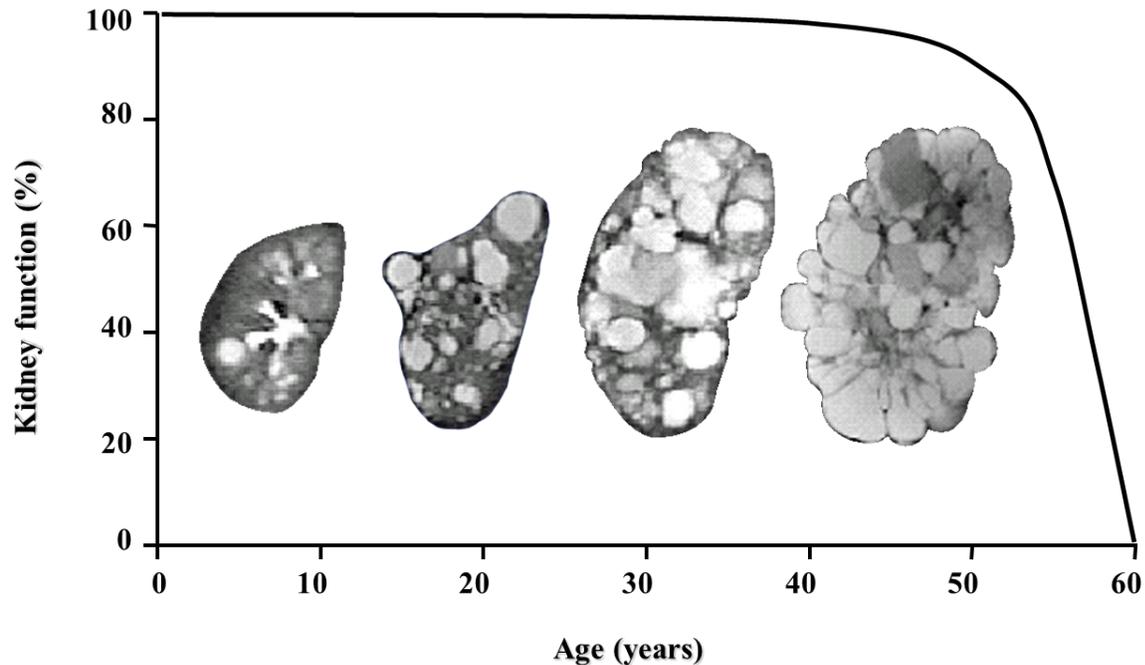
Courtesy V. Torres (Mayo Clinic)

All of the common signs and symptoms indicative of ADPKD progression are associated with increased TKV, including severe pain, increasing abdominal girth, hypertension, gross hematuria, nephrolithiasis, urinary tract infections, cyst hemorrhage, and kidney infection. TKV increase reflects a process that causes problems early on, which is reflected in hypertension, decreased concentrating capacity, and renal complications including gross hematuria, pain, and nephrolithiasis {Grantham 2011a}. On the other hand, the relationship between most clinical symptoms of ADPKD and GFR are highly variable. The manifestation of clinical symptoms relies on the overall structure, size, and organization of the organ, while GFR is maintained by the many-fold redundancy of the nephron units. Thus, GFR usually remains at or near normal until kidneys grow to approximately five-fold normal size {Grantham 2006a}. Beyond redundancy, the persistent stability of GFR is supported by hyperfiltration of surviving nephrons until they themselves become damaged or overwhelmed by chronic stress. The finding of stable GFR when ADPKD kidneys are dramatically enlarged and distorted by multiple cysts and fibrosis can provide a false reassurance regarding stability of disease progression. Inevitably, GFR declines at a rate of 4-6 ml/min/1.73m^2/year once renal insufficiency has developed, which is faster and more uniform than in other progressive renal disorders {Klahr 1995}. Evidence indicates that the mass of functioning renal parenchyma decreases significantly before changes in GFR are detected {Grantham 2011a, Meijer 2010}.

Given the absence of any regulatory-approved treatment therapies, sponsors are reluctant to invest in developing potentially promising compounds in the absence of a clear and viable regulatory path for clinical trial design. Accepted regulatory endpoints for clinical trials designed to slow progression of

chronic kidney disease are presently limited to development of kidney failure requiring renal replacement therapy and doubling of serum creatinine (sCr) {Levey 2009}. Because progression of ADPKD occurs over many decades, use of such endpoints would require studies to focus on late-stage disease, a stage when patients are not likely to respond to an intervention. When using subjects likely to benefit from therapy which could slow disease progression, the requirement to reach kidney failure as an outcome means that the time frame for performing a clinical trial (particularly with an earlier intervention) could be a decade or more, beyond the resources of any federal or private entity. Consequently, clinical trials in ADPKD are hampered by the lack of accurate, reproducible, reliable, quantifiable, easily measured biomarkers that correlate well with disease progression. Targeting therapies to the formation and early growth of cysts before major damage is done as opposed to targets aimed at the secondary effects of cysts (interstitial inflammation, fibrosis) requires early intervention.

Changes in kidney volume can be detected in early childhood {Fick-Brosnahan 2001}, and kidney volume exponentially increases with aging {Grantham 2006a, Grantham 2006b}. Additional recent evidence confirms that there are many more cysts in ADPKD kidneys than can be detected by the most sensitive MR methods used clinically {Grantham 2012}. TKV includes the volume of all the cysts indicating that the rate of change represented by serial TKV measurements reflects the enlargement of the cysts.

The strong association between TKV and renal function, the predictive power of TKV for the development of future renal insufficiency, and the association between TKV and other renal complications, therefore, make this an appropriate biomarker to consider for use in clinical trials in ADPKD.

The NIH-sponsored Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) study has documented the rate of kidney volume progression in ADPKD and demonstrated that GFR progression to Kidney Disease Outcomes Quality Initiative – Chronic Kidney Disease (KDOQI-CKD) Stage 3 is predicted by increased TKV. TKV of >1500ml (~five-fold normal), particularly in those younger than 30 years, was the primary predictor of GFR decline over an initial three-year observational interval in patients with initially preserved kidney function {Grantham 2006a}. This cohort's further follow up (CRISP II) for an additional five years has recently been published {Chapman 2012}, and supports the concept that initial TKV provides a sensitive and specific predictor of an individual's risk of developing CKD Stage 3 within eight years and other complications of ADPKD.

Despite this carefully monitored data set over eight years, the subjects (n=241; mean age 32.4 years) in CRISP I and II have not yielded sufficient numbers of ESRD events (n=24 after 10 years), hospitalizations, or deaths to allow construction of a disease analysis model that links to those categorical events. The PKDOC has, therefore, combined the longitudinal data from 2355 patients collected over more than 40 years, that includes thousands of longitudinal measurements of TKV, kidney function, and patient outcomes from several sources including well-characterized ADPKD disease registries at Emory University, Mayo Clinic, and the University of Colorado, as well as CRISP I and II. Data from these longitudinal, well-characterized observational registries maintained by leading PKD investigators at prominent American academic medical institutions were utilized. There were no specific exclusion criteria except for the CRISP observational cohort, and we believe that these registries are representative of the overall ADPKD population.

In the early stages of mapping the available datasets, there was an initial effort to map all existing PKD registry patient data (i.e., the only inclusion criterion was 'any subject with a diagnosis of ADPKD'). However, given the specific context of use to qualify TKV as a prognostic biomarker, it was decided that the inclusion criteria would be refined to 'any subject with a diagnosis of ADPKD and at least one available kidney volume measurement.' Prior to this refined inclusion criteria, some subjects without image data were mapped. Consequently, from the total population of 2610, 255 subjects have no imaging data and were excluded from analyses. Most of these subjects were from the Colorado registry and participated in two different ways. One group joined the registry after ESRD primarily for genetic analysis. The other group consisted of African American patients from the U. of Alabama provided by another investigator; these subjects were never seen at the U. of Colorado site. A disproportionate mortality rate in the total population was accounted for by subjects with no measurements of TKV and who had already reached ESRD at the time of joining the registry. Compared to subjects with at least one image, the subjects without an image tend to be older and from an earlier time period.

Given the proposed context of use, the team has focused on the 2355 subjects for whom TKV measures are available. The 255 subjects without TKV measurement are not relevant to the assessment of TKV as a prognostic biomarker, and these subjects were not included in any analyses. Figure 4 provides an overview of the study populations.

Figure 4: Data Characterization for Primary Study Populations
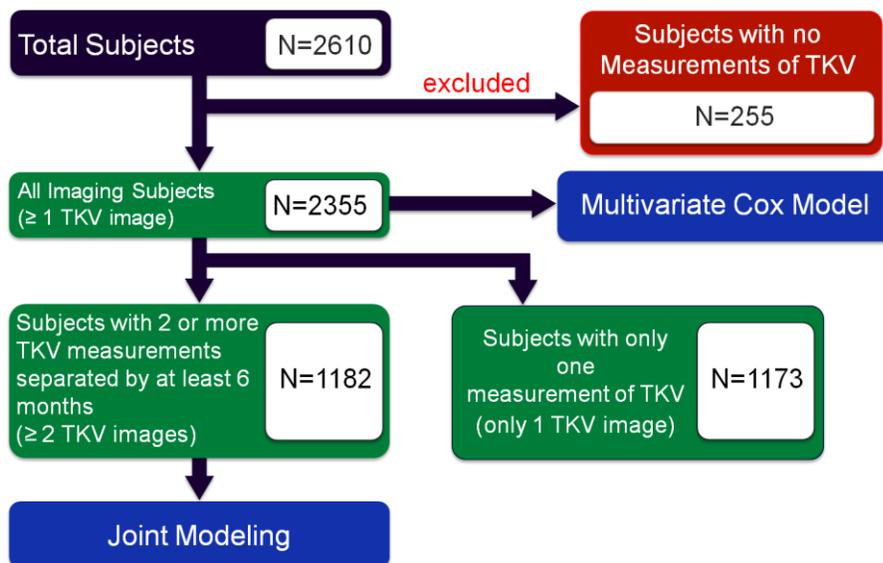
Among the 2355 subjects with at least one measurement of TKV (referred to as **"≥1 TKV"**), approximately half, or 1182, had two or more measurements of TKV at least six months apart. Those 1182 subjects with two or more measurements of TKV (referred to as **"≥2 TKV"**) allow the joint modeling of TKV, age, and the endpoints of interest. The characteristics of the two subgroups are shown in Table 1, and they are quite similar. The younger age and lower mortality of the ≥2 TKV measurements subjects is accounted for by a large component (~25%) made up by the CRISP population, who were selected for younger age and preserved kidney function.

A comparison of age at death and ESRD in patients with ≥1 TKV and ≥2 TKV is shown below. The age of ESRD (~52) is similar to the age of ESRD reported by the USRDS: 55-56 years of age. (See **Table 1**)

Table 1: Characteristics by Imaging Population

Characteristic	At Least One Image (n=2355)		
	At Least One Image (n=2355)	Only One Image (n=1173)	Two or More Images[1] (n=1182)
Age at Study Entry (Years), *mean ± SD*	35.37 ± 16.28	39.31 ± 15.74	31.45 ± 15.86
Year of Study Entry, *mean ± SD*	1995 ± 11.67	1997 ± 11.08	1993 ± 11.87
Sex (Male), *n (%)*	933 (39.62)	479 (40.84)	454 (38.41)
Death			
Count, *n (%)*	145 (6.16)	90 (7.67)	55 (4.65)
Age at Death, *mean ± SD*	62.33 ± 14.50	62.55 ± 13.37	61.97 ± 16.30
End Stage Renal Disease (ESRD)			
Count, *n (%)*	546 (23.18)	309 (26.34)	237 (20.05)
Age at ESRD, *mean ± SD*	52.27 ± 10.57	52.28 ± 10.48	52.27 ± 10.70

"Two or More Images" subjects have two or more renal images > 6 months apart

To enable an appropriate analysis of the data, the PKDOC has developed common data standards in collaboration with CDISC to which these data have been mapped. Applying the data standards has facilitated the aggregation of significant amounts of untapped longitudinal PKD data into a common database. This is the first database to employ disease-specific CDISC SDTM (Study Data Tabulation Model)-mappable standards for the data elements needed for ADPKD.

The PKDOC has utilized quantitative modeling tools to support the qualification of TKV as a prognostic imaging biomarker. The FDA has recognized quantitative modeling tools as significant areas of interest (link: FDA Pharmacometrics 2020 Strategic Goals).

As such, the field of pharmacometrics has evolved significantly to provide quantitative tools that can improve the drug development process {Romero 2010}. In order to generate the necessary evidence to support the qualification of TKV as a prognostic imaging biomarker, the PKDOC has developed a series of sequential models, leveraging previously published work around the progression of TKV change over time. The PKDOC has also quantified the relationship between baseline TKV (in combination with baseline eGFR and patient age) and clinically relevant endpoints that track the progression of ADPKD.

The PKDOC originally looked at six potential endpoints: onset of hypertension; transition from Chronic Kidney Disease stage 1 or 2 to stage 3 or higher; 30% worsening of eGFR; 57% worsening of eGFR; ESRD; and mortality. Based on the available data, and the results of the modeling and analysis, this

effort will show the strong correlation of baseline TKV to the loss of kidney function in ADPKD measured by the worsening of renal function (30% and 57% decline in eGFR), and the development of ESRD. The other three endpoints were dropped. For the previous analysis of these endpoints see Appendix 8.5

3.2   Regulatory Background

3.2.1   Summary of Previous Regulatory Interactions

PKDOC was launched in July, 2010, following discussions between the PKD Foundation, Dr. Ronald Perrone, and the FDA. A Voluntary eXploratory Data Submission (VXDS) meeting was held with the FDA on November 18th, 2011. Discussions with the EMA were initiated in January, 2013. A summary of the interactions with the regulatory agencies can be found below in **Table 2**.

Table 2: Summary of PKDOC Regulatory Interactions

Date	Event	Summary
7/17/2007	FDA and PKD Foundation Workshop: Clinical Trial Endpoints in Polycystic Kidney Disease. Co-chairs Drs. Perrone and Guay-Woodford.	Acceptance of kidney/cyst growth as a primary outcome will facilitate interest of biopharmaceutical industry in drug development in PKD
3/27/2008	PKD Database Consortium Meeting led by Dr. Perrone.	Establishing PKD clinical database to (1) aggregate data across registries and clinical trials; and (2) simulate clinical trials to detect disease progression or symptom relief.
1/28/2009	Teleconference with 39 participants including FDA, CDISC, C-Path, PKDF, NIH, clinicians, Amgen, Genzyme, Otsuka, Novartis, Roche, Wyeth, LC Pharma, Cystonix.	Provided overview of PKD Foundation and CDISC. Determined interest of key stakeholders. Strategized funding and next steps.
8/27/2009	Face-to-Face PKD Consortium meeting. Facilitated by Dr. Perrone and CDISC. Attended by FDA, C-Path, PKD Foundation, and industry members.	Launched the effort to create an SDTM standard for the common PKD data elements to provide foundation for mapping legacy and prospective data. Included a discussion on the biomarker qualification process, the value of prospective data, and the modeling strategy. Support from FDA to develop common PKD data elements. Decision to formalize PKDOC with C-Path.
12/3/2009	Face-to-Face PKD Consortium meeting (held at Otsuka office). Facilitated by Dr. Perrone. Attended by FDA, CDISC, C-Path, and industry members.	Continued progress on defining the common ADPKD data elements and the STDM mapping efforts.
7/15/2010	Teleconference	Official launch of PKDOC.

Date	Event	Summary
8/24/2010	Face-to-Face PKDOC workshop. Facilitated by Dr. Perrone. Attended by FDA, CDISC, C-Path, and industry members.	Prioritized common ADPKD data elements. Dr. Stockbridge (FDA) provided additional information on possible qualification outcomes.
12/1/2010	Face-to-Face meetings with FDA and C-Path.	Overview of PKDOC and activities to date. Request for official FDA Liaison for PKDOC.
3/29/2011	Teleconference with FDA (Drs. Pendse, Walton, Hills, and Thompson) to provide overview of PKD, and to discuss pre-submitted questions regarding disease rating scales, disease modeling approaches and key considerations for TKV as imaging biomarker for qualification.	FDA discussed Context of Use statement as key to BM qualification submission. Concluded that PKDOC will prepare initial briefing package and that VXDS meeting with FDA would be evaluated. Another review session to be scheduled with FDA.
7/1/2011	Planned submission of Letter of Intent (LOI). FDA feedback from Dr. Walton that PKDOC not ready for LOI – required VXDS meeting first.	LOI deferred until after VXDS meeting.
7/21/2011	Face to Face meeting with Dr. Dennis at FDA with Drs. Walton, Hills, Pendse, and Thompson.	Discussion included Context of use statement, caution regarding terminology of "efficacy endpoint". FDA suggested Subpart H route could be explored. FDA also discussed possibility of a disease rating scale. FDA recommendation to seek FDA input through VXDS process. FDA assessment that consortium not ready for LOI and formal BQRT engagement.
10/14/2011	VXDS Briefing Book submitted by PKDOC to FDA.	Initiated formal FDA review process in preparation for the VXDS meeting scheduled for Nov 18, 2011.
11/18/2011	VXDS Face-to-Face meeting with FDA and PKD Outcomes Consortium, chaired by Dr. Amur (FDA), and facilitated by Drs. Dennis and Perrone (PKDOC).	FDA support to proceed to formal biomarker qualification Letter of Intent to FDA. FDA support for qualification of TKV as a prognostic biomarker for patient selection for clinical trials. FDA encouraged PKDOC to provide comprehensive review of literature with regards to pathophysiology of PKD and results of animal studies, and stressed importance of patient data with two or more images. PKDOC developed and distributed minutes to participants.

Date	Event	Summary
1/3/2012	PKDOC submits Letter of Intent to FDA	Context of Use submitted: Baseline TKV can be applied as a prognostic biomarker that, in combination with patient age and other covariates, can accurately predict the risk and cadence of disease progression in ADPKD patients. As such, baseline TKV can be applied as a biomarker to enrich clinical trial populations with patients most likely to demonstrate a response to, and a benefit from, therapeutic interventions.
2/1/2012	PKDOC receives official acceptance of Letter of Intent and submission into the Biomarker Qualification Program.	Submission in "Stage 1: Consultation and Advice" of the qualification process. A Qualification Review Team (QRT) was formed to further assess the LOI and an internal meeting of this QRT was planned to provide list of topics/issues the QRT would like to see addressed in an Initial Briefing Package submission.
3/6/2012	PKDOC receives feedback from BQRT regarding TKV qualification and Briefing Book recommendations.	Feedback detailed separately below.
4/13/2012	Teleconference with FDA (Drs. Walton and Pendse) to review key PKDOC questions (provided in advance).	Questions: 1. Combining Colorado and CRISP 1 datasets for preliminary analysis; (After the discussion, it was decided that no preliminary analysis would be done on the Colorado or CRISP datasets). 2. FDA current thinking on qualification of disease models. 3. Review of objectives of Face-to-Face meeting to review briefing book.
9/24/2012	PKDOC submits the Briefing Book to the FDA.	Submission acknowledged by the FDA, and the Face-to-Face BQRT meeting date was finalized for November 9th, 2012.
11/2/2012	Preliminary comments on the Briefing Book received from the BQRT.	Initiated detailed PKDOC preparation for the November 9th BQRT (presentation content and meeting logistics).
11/9/2012	PKDOC / FDA BQRT Face-to-Face meeting held at the White Oak campus in Maryland.	Reviewed all comments received from the BQRT with focus on the data content and the statistical analysis plan. PKDOC was in agreement with the FDA recommendations, and a follow-on teleconference was planned to address a remaining analysis methodology question.

Date	Event	Summary
12/5/2012	PKDOC / FDA BQRT follow-up teleconference held.	Resolution of the analysis methodology question was achieved and BQRT agreed with plans to proceed with the analysis. The data content was determined to be adequate for qualification consideration. It was also announced that Dr. Shona Pendse, the FDA Liaison to the PKDOC, would be leaving for another assignment.
12/20/2012	PKDOC submitted their unofficial minutes for the November 9th, 2012 meeting.	Receipt acknowledged by the FDA with note that the official minutes would be provided by the FDA.
2/13/2013	Official BQRT minutes received from the FDA.	The minutes for both meetings (Nov 9th and Dec 5th) were combined into a single document. Receipt of the minutes was acknowledged by PKDOC.
4/10/2013	LOI submitted to the EMA	Accepted.
4/30/2013	Briefing Books submitted to the EMA and FDA	Accepted by both agencies.
6/13/2013	Received List of Questions on the Briefing Book from the EMA.	Scheduled and initiated preparation for Face-to-Face meeting.
6/20/2013	Received Questions on the Briefing Book from the FDA	Scheduled and initiated preparation for Face-to-Face meeting.
6/28/2013	Face-to-Face BQRT meeting held with FDA in Silver Spring, MD.	Reviewed all comments and questions that had been submitted by the FDA on 6/20. Seeking approval to submit final briefing book.
6/28/2013	Submitted written response to the EMA on their List of Questions	Addressed all questions raised in the EMA document received on 6/13.
7/9/2013	Face-to-Face SAWP meeting held with FDA in London.	Reviewed all questions that had been submitted by the FDA on 6/20. Seeking approval to submit final briefing book.
7/15/2013	PKDOC submitted minutes of 7/9 meeting to the EMA	EMA requests that the submitter provide minutes. Also shared with the FDA.
7/19/2013	Follow-up teleconference with the FDA.	Purpose was to review FDA questions in two areas: relationship between eGFR and TKV, and TKV's prognostic value for the PKD population most likely to be enrolled in clinical trials (i.e., those with preserved eGFR).
7/24/2013	Received list of additional questions from the EMA.	Approved submission of final Briefing Book and requested that the Consortium address the latest questions in the form of an updated Briefing Book.
7/26/2013	PKDOC submitted response to information requested at 7/19/2013 teleconference.	Provided information related to the two questions. FDA accepted the information and indicated they would provide a response.

Date	Event	Summary
8/15/2013	Received summary minutes from the FDA.	Official minutes that covered the 6/28 BQRT meeting and the 7/19 follow-up teleconference.
8/29/2013	Additional follow-up teleconference held with the FDA.	FDA provided feedback on the 7/26 PKDOC response and asked what it would take to do a re-analysis that includes eGFR as a covariate. PKDOC was given time to prepare for a final follow-up TC.
9/18/2013	PKDOC submitted advance document with requested information regarding eGFR	Document received and reviewed by the FDA in preparation for 9/24/2013 teleconference.
9/24/2013	Follow-up teleconference held with the FDA to review the PKDOC response.	Purpose of the meeting was to review FDA questions on the materials provided on 9/18.
9/27/2013	Response received from the FDA on 9/24 presentation	FDA approved submission of the final Briefing Book with a list of questions that should be addressed.

3.2.2   Summary of Regulatory Comments and Recommendations

Table 3 below provides a summary of regulatory comments and recommendations, and the corresponding section of this document that addresses that topic.

Table 3: Summary of Regulatory Comments with Reference to PKDOC Responses

	Regulatory Recommendation/Comment	Source	Response
1.	Please discuss the range of endpoints with which you plan to assess PKD disease progression and show that total kidney volume (TKV) is prognostic for disease worsening in these endpoints, (e.g., slope of GFR or progression to specific CKD stages), your reasons for choosing those endpoints, and provide a detailed description.	March 2012 FDA LOI Comments	Sections **3.4.6**, **5**, and **6**.
2.	Please also describe the quantitative approach of how you propose to perform the comparison of baseline TKV to the endpoints.	March 2012 FDA LOI Comments	Sections **4** and **5**
3.	Please describe what is currently known regarding correlations of rate of symptom progression and increase in TKV from available data. Include any other evidence (other trials such as the sirolimus or everolimus trials, etc.) which provide evidence for and against use of TKV, and any explanations that you think may account for the outcomes.	March 2012 FDA LOI Comments	Sections **3.6** and **3.7**
4.	We anticipate the need for a comprehensive review of the literature that describes the natural course of the disease as assessed by various clinical measures including specific imaging modalities. It will be best if you develop and propose a detailed plan for how you will:	March 2012 FDA LOI Comments	Section **3.7**

	Regulatory Recommendation/Comment	Source	Response
	a. Conduct a systematic search of the literature b. Select articles for review and summary c. Perform descriptive and if warranted, formal analyses. Your proposed systematic literature review methodology for the natural history for autosomal dominant PKD is adequate. However, you should also provide a detailed list of instances in which the conclusions of the two investigators who reviewed the data differed, and also the documents which detail the ultimate decision making process by all of the Principal Investigators of the PKDOC.	Nov 2012 Preliminary BQRT Comments	Appendices 8.3 and 8.4
5.	Please provide information on the following, in your briefing document: a. Natural progression of the disease b. Method of diagnosis and typical age at diagnosis c. Current standard of care (and variations there in) d. Summary of interventional and observational studies previously conducted in this population. e. Animal models	March 2012 FDA LOI Comments	Sections **3.4.2**, **3.4.3**, **3.4.4,** and 3.4.5 for (a), (b), and (c) Section **3.6** for (d) Section **3.5** for (e)
6.	Please provide a summary of the key image acquisition and reconstruction parameters for each imaging modality. Include a description of: a. Settings for each imaging modality, e.g. Hz for US; kV, mAs, reconstruction settings for CT; pulse sequences and other acquisition parameters for MRI b. Acquisition of volume measurements, e.g., post-processing software, interactive or automated measurement tools, and methods used to validate measurements (such as phantoms) c. Assessment of test and reader performance, e.g., variability. To the degree you are able, please separate intra-patient variability in the primary imaging data, inter- center variability due to the different devices or device method of use within each modality, intra and inter-reader variability.	March 2012 FDA LOI Comments	Section **4.3.2**
7.	Please provide a detailed description of the registries of images and other patient data that you have collected. Include the following information: a. A summary of the clinical protocol. b. Number of patients, patients' disposition, duration of follow up, and missing data c. Listing of clinical data elements d. Clinical variables for the data	March 2012 FDA LOI Comments	Sections **4.1** for (a) and (b) Appendix 8.2 for (c) and (d)

	Regulatory Recommendation/Comment	**Source**	**Response**
	e. Ongoing and planned analyses of data. Please clarify whether you plan to evaluate the contribution of the covariates with as well as without the baseline TKV considerations in the prognosis of the risk and cadence of disease progression in ADPKD patients.		Sections **4.5**, **4.6**, and **5** for (e)
8.	Include a description of the variables in the CDISC PKD data standards.	March 2012 FDA LOI Comments	Appendix 8.2
9.	Please elaborate on your plan for handling missing data.	March 2012 FDA LOI Comments	Described in each endpoint analysis topic in Section 5. Addressed also in Section **4.4** and Appendix 8.1
10.	Please provide graphical representation of time courses of TKV, markers of renal function, and other pharmacodynamics markers for the studies where you currently have access to the data. Please present these as absolute values over time and also as change from baseline over time. In addition, present subsets with marked changes in TKV over time separately. Where applicable, please present time course data as quartiles of baseline TKV (e.g., TKV measurement in CRISP).	March 2012 FDA LOI Comments	See Appendices 8.9 and 8.10
11.	If you include any figures in the briefing package, please provide a clear description of what the figure is showing. If the figure shows means and confidence intervals, include the number of subjects on which those estimates are obtained.	March 2012 FDA LOI Comments	Throughout
12.	You have described multiple rodent models of PKD. Are there any non-rodent models that may also be considered? If so, we are interested in hearing a description of them.	Nov 2012 Preliminary BQRT Comments	No non-rodent models are being considered. Mutations in polycystin 1 resulting in typical polycystic kidney disease have been reported in Persian cats. See Section 3.5
13.	You should plan to provide complete adjudication packets for all the cases of conflicting data which necessitated adjudication of clinical events, such as instances in which there was a conflict between the clinical events recorded in the registries for a given subject and the same events recorded in CRISP as medical history for that particular subject.	Nov 2012 Preliminary BQRT Comments	Section 4.1.5 and Appendix 8.1.
14.	If you plan to seek qualification of TKV as prognostic for endpoints not part of the current usual efficacy	Nov 2012 Preliminary	Section 3.3 Also please reference

	Regulatory Recommendation/Comment	**Source**	**Response**
	endpoints, you should plan to explain how this approach will aid drug development. If you plan to propose that TKV would be used in a Phase 3 study with an efficacy endpoint that is not among the current usual endpoints, please plan to discuss why the different endpoint would be an appropriate efficacy endpoint for drug approval.	BQRT Comments	the NKF/FDA Scientific Workshop, "*GFR Decline as an Endpoint for Clinical Trials in CKD*" meetings held Dec 3-4, 2012. Note: At this time, we are only seeking qualification of TKV as a prognostic biomarker for use in drug development to enrich clinical trial populations.
15.	You should plan to provide clear descriptions of the value of TKV as an enrichment factor. For example, a table(s) showing expected rates of study endpoints (and confidence intervals around those estimates taking into account uncertainties from the modeling process) for specific TKV criteria (or algorithm) in hypothetical study designs where important design parameters such as study size, duration, power, specific endpoint are also shown will aid in understanding the value of TKV in drug development. For each different case, the same information for a comparison hypothetical study without use of TKV will be helpful.	Nov 2012 Preliminary BQRT Comments	Sections 5 and 6
16.	The value of TKV in drug development should be examined and illustrated for each method of using TKV you intend to propose for qualification (e.g., single baseline measurement, longitudinal change). Please ensure the TKV criteria and any other essential eligibility criteria that affect the prognostication are fully specified for each illustration of use in a hypothetical study.	Nov 2012 Preliminary BQRT Comments	Section 3.3. Note that only baseline TKV is being proposed for qualification in this document.
17.	We understand from your briefing package that you will be deciding whether to model TKV using US data only, MRI/CT data, or modality-independent data (US, CT, and MRI) calculated by use of a scaling factor. Regardless of which of these you decide ultimately to use, please provide analyses based on each of the previously listed datasets (as sensitivity analyses).	Nov 2012 Preliminary BQRT Comments	Sections 4.3 and 5
18.	In addition to the methods for controlling variability, please describe if/how the potential bias in volume measurements was controlled (e.g., whether or not the readers were blinded to clinical data and whether for longitudinal studies the images at each time point were	Nov 2012 Preliminary BQRT Comments	Section 4.1 All data are from long-term registries or an observational cohort (CRISP1 and 2)

	Regulatory Recommendation/Comment	Source	Response
	presented in a random, independent fashion). In the briefing document, the exponential growth model for TKV does not have an interpretable solution at Time=0. The left-hand side needs to be an absolute value of TKV rather than change from baseline in TKV (ΔTKV). If you are planning to quantify change, the equation needs to be corrected accordingly. We had provided this comment for the LOI, but the comment has not been addressed in the briefing document.		without intervention. Readers were not aware of clinical data. Images were obtained when patients presented for follow-up, not on a regular or predictable interval (except in the CRISP1 and 2 cohorts).

Based on analysis results, the exponential model is no longer being used. A linear model is used with a log transformed value as described in Section 5. |
| 19. | We recommend splitting the data into two halves: a training set and test set. The joint model including any model selection should be done independently on the training set. Possible covariates and models should be pre-determined as well as the criteria for choosing the best model. After the best model is chosen from the training set, the fitted model should be tested with the remaining half of the data. You are advised to consider and describe the implications of restricting data from subjects with at least two TKV measurements for the model selection and validation process (compared to using all subjects including those with only a single TKV measurement). | Nov 2012 Preliminary BQRT Comments

Nov/Dec 2012 BQRT Meeting Minutes | Section 4.1.6 Due to the relatively low sample size in some modality specific datasets, a different approach using a five-fold process was agreed upon at the Dec 5, 2012 teleconference.

Sections 4.6 and 5. Patients with at least two TKV measurements were used for the Joint Modeling. For patients with single TKV measurements, multivariate Cox models were used to evaluate the effect of baseline TKV (as well as other factors). |
| 20. | Given that the goal is to predict long-term clinical events based on early biomarker data, it is better to avoid using predicted longitudinal biomarker as a time-dependent covariate in a survival model unless there are long term longitudinal biomarker data to quantify the | Nov 2012 Preliminary BQRT Comments | Sections 4.6 and 5 |

	Regulatory Recommendation/Comment	**Source**	**Response**
	biomarker model for reliable long term biomarker prediction. Covariates that can be quantified reliably based on the early biomarker data such as baseline biomarker level or an initial slope can be used as time-independent covariate to avoid the concern about long term biomarker prediction.		
21.	We recommend that you consider exploring the use of MRI-based volume estimates alone for prognosis (in addition to your plan to combine CT and MR volumetric measurements). It will be important to us to understand the consistency of the predictions when based on MRI or CT to judge the appropriateness of general use of either modality.	Nov 2012 Preliminary BQRT Comments	Sections 4.3 and 5. CT and MRI TKV measurements have been shown to be virtually identical.
22.	Please clarify the number of subjects with two or more images using the same modality that also had an endpoint of ESRD/death.	Nov 2012 Preliminary BQRT Comments	Each endpoint section provides these details. Please reference Sections 5.3 and 5.6 and Appendices 8.15 and 8.17 from the BB submitted on 4/30/2013.
23.	When analyzing TKV with an ROC curve, we recommend that you use methods for time-dependent ROC curves. See (*Heagerty, P., Lumley, T., and Pepe, M. (2000). Time-dependent ROC curves for censored survival data and a diagnostic marker. Biometrics 56, 337–344.) and (Cai, T., Gerds, T. A., Zheng, Y. and Chen, J. (2011), Robust Prediction of t-Year Survival with Data from Multiple Studies. Biometrics, 67: 436–444.)*	Nov 2012 Preliminary BQRT Comments	Sections 4.6 and 5. In addition to ROC curves, Joint Modeling and Cox Proportional Hazard Models were used to thoroughly analyze the TKV relationships.
24.	To demonstrate that baseline TKV improves diagnostic accuracy, you should consider a composite risk score using only other covariates versus a composite risk score including baseline TKV. 'Other covariates' should be pre-specified.	Nov 2012 Preliminary BQRT Comments	Analysis based on available data show that age and baseline eGFR are the best covariates. See section 5.
25.	When analyzing the relationship between longitudinal TKV measurements and time to event (ESRD or death), simultaneous modeling would be better (see the review paper, *Joint modeling of longitudinal and time-to-event data: an overview Tsiatis, A. A. Davidian, M. STATISTICA SINICA 2004, vol 14; part 3, pages 809-834*). We agree with the statement "naive approaches to inference on relationships between longitudinal and time-to-event data are inappropriate" in the Discussion section of the paper.	Nov 2012 Preliminary BQRT Comments	Simultaneous modeling was used. Sections 4.6 and 5.
26.	We recommend that you develop a detailed analysis	Nov 2012	Reference the imaging

	Regulatory Recommendation/Comment	Source	Response
	plan for comparing the reliability of the various methods of TKV measurement and for establishing specifications for the measurement method you will select for determining the relationship between TKV and disease outcomes.	Preliminary BQRT Comments	sections for Mayo, Emory, and Colorado (Section 4.3)
27.	Please note that an eventual biomarker qualification submission will need to include complete summaries of the studies you have performed to verify the reliability of the TKV measurements.	Nov 2012 Preliminary BQRT Comments	No additional studies have been performed. See Section 3.6 for summary of existing studies, and Sections 4.6 and 5 for the model validation process.
28.	To aid in understanding the breadth and depth of the data you use in these analyses, please plan to include displays of the data (e.g., histograms, tables) describing the amount and time-frame for data of each type used in the analyses. In addition to displays of variables such as age at entry, year of study entry, etc., shown in your briefing document, this can include displays of all factors that may have an important influence on the analysis results, such as histograms showing the numbers of patients with differing lengths of follow-up after the TKV measurement in each dataset used in modeling, and for each endpoint modeled. The numbers of patients with missing data should also be represented. Please plan to distinguish between patients with PKD-1 and PKD-2 mutations, or any other intrinsic characteristics that might influence the generalizability of the analysis results. The numbers of patients with repeat TKV measurements (if any), by the time period between measurements may also be informative.	Nov 2012 Preliminary BQRT Comments	These type of data are provided in Section 4.2, within each endpoint analysis section (5.1 through 5.3), and in Appendices 8.9 through 8.10.
	Fully characterize the Subjects with two or more images to demonstrate if they are generalizable.	PKDOC BQRT Minutes	Section 4.2 and each endpoint analysis topic. Section 5
29.	You are seeking to qualify TKV as a patient selection factor for clinical trials. Qualification is appropriate for prognostic factors that can be understood to have a valuable role in improving drug development over drug development without use of the factor. We are interested in your perspective of what measure of utility would be best to consider, the magnitude of utility on that measure that shows it is valuable, and level of confidence in that estimate would be sufficient to support qualification for this context of use.

In general, for Phase 3 studies, prognostic markers are | Nov 2012 Preliminary BQRT Comments | Please see reference Section 3.3 for an example of how TKV will be used in the drug development process as a prognostic biomarker. |

	Regulatory Recommendation/Comment	**Source**	**Response**
	typically used to increase the statistical power for demonstrating a treatment effect on the primary endpoint of the study. Please plan to discuss how TKV would be used and the impact on sample size for a desired power (or study power for a specified sample size) as compared to not using TKV.	Nov/Dec 2012 BQRT Meeting Minutes	
30.	A clarified Context of Use statement well-aligned with the data intended to support the context of use is valuable.	Nov 2012 Preliminary BQRT Comments	Section 3.3
31.	While viewing the planned table PKDOC will use for demonstrating a relationship between baseline TKV and clinical outcomes, FDA stated that to demonstrate the value of TKV, the Submitter will have to incorporate an understanding of other important covariates. The Submitter agreed to this.	Nov/Dec 2012 BQRT Meeting Minutes	Section 3.3. The other covariates of interest are age and baseline eGFR and are included in tables as appropriate.
32.	The mortality rate for "All Subjects" is ~10%, while that for the "Subjects with two or more images" is ~ 5%. What accounts for this difference? FDA stressed that it will be important for the Submitter to describe how the subjects in the "Subjects with two or more images" dataset differ from the "All Subjects" dataset.	Nov/Dec 2012 BQRT Meeting Minutes	Section 3.1 (page 18)
33.	FDA asked the Submitter if there were any summary statistics looking at GFR. PKDOC replied that they will get back to FDA with this information. FDA replied that this information should be included in the full qualification package.	Nov/Dec 2012 BQRT Meeting Minutes	The distribution of baseline eGFR is shown in Figure 22, Sections 5.1 and 5.2, and Appendix 8.10.
34.	[PKDOC is] in agreement with the FDA that the TKV model will be built using MRI/CT data with and without ultrasound data, and ultrasound data only. A sensitivity analysis will be performed by comparing rates of TKV growth.	Nov/Dec 2012 BQRT Meeting Minutes	Addressed within each endpoint analysis topic in Section 5.
35.	FDA stated that they would like to understand how much value TKV adds on top of other covariates and requested the Submitter to include relevant information in the full submission. Referring to the "Histogram for Disease Outcomes of Interest – Mortality and ESRD," FDA also asked the Submitter if the histogram for selected disease outcomes could be prepared for the "Subjects with two or more Images" dataset in the full qualification package as this would provide information about how TKV as a prognostic factor differs from TKV for treatment indication. PKDOC agreed with this request.	Nov/Dec 2012 BQRT Meeting Minutes	Histograms of baseline characteristics of patients with disease outcomes of interest were provided for "Subjects with two or more images". Sections 4.2 and 5.
36.	FDA then asked that the Submitter include a description of the datasets in their next briefing document submission to the FDA, along with the analysis results.	Nov/Dec 2012 BQRT Meeting	Sections 4.1, 4.2, 4.3, 4.5, and 5

	Regulatory Recommendation/Comment	Source	Response
	PKDOC agreed with this request.	Minutes	
37.	FDA noted that the five-fold cross validation explained allows more rigorous validation than the presented approach proposed by PKDOC. PKDOC replied that they will use FDA's recommended approach. Carefully describe the validation approach.	Nov/Dec 2012 BQRT Meeting Minutes PKDOC BQRT Minutes	Section 4.6 and within each endpoint topic in Section 5
38.	FDA requested PKDOC include their definition of "estimation on prediction accuracy" in their full qualification package submission when that point arrives. PKDOC stated they would do this. Validate with both observed and derived data to prove that derived predicts observed.	Nov/Dec 2012 BQRT Meeting Minutes PKDOC BQRT Minutes	Confidence intervals are provided for all endpoint analyses in Section 5.
39.	[It is not clear] whether a model to predict TKV growth is better, or measuring TKV to estimate TKV growth is better. We ask that you provide a detailed description of your joint modeling approach in your full qualification submission. It is crucial to obtain the right prediction. Thus, we also want you to measure TKV growth using TKV values. We will then compare the results derived using the two approaches.	Nov/Dec 2012 BQRT Meeting Minutes	Based on the data analysis, the TKV growth model was not pursued. Section 4.5 and 5
40.	FDA: Will you also consider using collected TKV baseline data from patients without modeling?	Nov/Dec 2012 BQRT Meeting Minutes	There is no external dataset available at this time for validation. The five-fold process was utilized to address this issue. See Section 4.6.
41.	FDA: How many subjects had more than two TKV measurements over time? PKDOC: We will provide this information to you in our full qualification submission.	Nov/Dec 2012 BQRT Meeting Minutes	See **Table 13**
42.	Provide data to illustrate the number of measurements per subject and the duration between measurements.	PKDOC BQRT Minutes	Section 4.3, **Table 13**and **Table 14**
43.	Longitudinal Change TKV vs. Rate of TKV growth. What is the difference between the two BMs? Is it only a different parameterization? The intended application is too broad. More specific claims will help focusing the discussion. Also, examples will help the Qualification Team understand how you are planning to use your BMs.	EMA email dated 4/12/2013	Longitudinal Change TKV and Rate of TKV growth are NOT a part of this submission. Only Baseline TKV is being submitted for qualification as a

	Regulatory Recommendation/Comment	Source	Response
			prognostic biomarker. Section 3.3
44.	Please clearly state the role of the TKV growth model, especially with respect to the intended applications in the Context of Use. For example, do you intend to use the TKV growth model as a placebo group in clinical trials?	EMA email dated 4/12/2013	TKV growth model is NOT being submitted in this application. Only Baseline TKV is being submitted for qualification as a prognostic biomarker. Section 3.3
45.	Please make sure that the different model assumptions are clearly stated. How are these supported by literature and in-house data?	EMA email dated 4/12/2013	Please see sections 3.7, 3.8, 4.6, and 5.
46.	Is the validation approach limited to diagnostic plots?	EMA email dated 4/12/2013	No, reference sections 4.6 and 5.
47.	Please clearly state the role of the Biomarker-Disease model, especially with respect to the intended applications stated in the Context of Use. For example, is it the model or the BM that will be used in the clinical trials? Will the baseline TKV be used as an enrichment BM to define a population most likely to respond?	EMA email dated 4/12/2013	Only Baseline TKV is being submitted for qualification as a prognostic biomarker. The supporting model itself is not being submitted for qualification. Section 3.3
48.	Will the TKV disease model be used to fill in the gaps regarding missing TKV data?	EMA email dated 4/12/2013	No, there are no imputed values. Section 4.4
49.	Eight different clinical outcomes are described in the 9/24/2012 Briefing Book. Will you develop eight different BM-disease models? What happens if the outcomes point to different directions regarding the performance of the BM?	EMA email dated 4/12/2013	Section 3.4.6. Six clinical outcomes were analyzed in this submission. A different model was developed for each, and Section 5 provides a conclusion on the performance of each biomarker.
50.	Please clearly state the role of the Longitudinal Change and Rate of Growth models, especially with respect to the intended applications stated in the Context of Use. For example, if TKV change is used to support PoC, dose finding, and confirmatory trials, what would be the change that needs to be demonstrated for the respective purpose and how long apart should the two measures	EMA email dated 4/12/2013	Only Baseline TKV is being submitted for qualification as a prognostic biomarker. Section 3.3

	Regulatory Recommendation/Comment	**Source**	**Response**
	be?		
51.	What is the role of htTKV with respect to the intended applications stated in the Context of Use? Why wasn't this parameter modeled?	EMA email dated 4/12/2013	The natural log of TKV without height adjustment will be used for biomarker qualification. Height-adjusted TKV did not add significant value to offset the potential practicality of use for clinical trial enrichment.
52.	Eight different ROC curves will be created. What if they demonstrate different prognostic performance?	EMA email dated 4/12/2013	Six (not eight) ROC curves have been created for six different clinical outcomes. In addition, Joint Modeling and Cox Proportional Hazard Models were used to thoroughly analyze the TKV contribution to the outcomes. See sections 4.6 and 5.
53.	Please provide summary questions for the EMA followed by a brief description of the sponsor's position, the supporting data, and the justification. Since you will have the data, your questions should focus on the interpretation of the data and the claims.	EMA email dated 4/12/2013	Section 3.2.3
54	Make all R programming code used for the modeling available to the FDA.	FDA F2F Meeting on 6/28/2013	This is provided as attachment with the final submission package.
55	Provide the same descriptive data for the analysis datasets (the 2355, 1182, and 1173 populations) as were provided for the full population.	FDA F2F Meeting on 6/28/2013	Please see section 3.1, page 18. Plots are in section 4.2.
56	Assess the impact of adding a confirming measurement of serum creatinine decline (30% or 57%) under a relaxed data rule. (New Rule: use the date that the subject crossed the threshold the first time, and use any subsequent measurement to confirm the decline. No confirmation interval requirement should be applied.)	FDA F2F Meeting on 6/28/2013	Data provided to the FDA in follow-up telecon of 9/24/13. The restrictive rule (requiring confirmation) is being used. See also Sections 4.4 and 5.
57	Relax the data rule on adult height measurements to see if enough data points are added to perform height-	FDA F2F Meeting on	Evaluation completed. Height-adjusted TKV

	Regulatory Recommendation/Comment	**Source**	**Response**
	adjusted TKV analysis. (New Rule: use any available height measurement for subjects over 18 years old.)	6/28/2013	did not add significant value to offset practicality of use in trial enrichment. Only TKV is used.
58	Add more explanation to address differences between populations used to develop the model, and populations that will use the model	FDA F2F Meeting on 6/28/2013	Provided in written response to FDA in July 2013. Also see Figure 4.
59	Provide Additional Details on the analysis a. Provide full details of every analysis step that generated tables, with full description of the filters that were applied to arrive at the 'N' for each analysis dataset. b. Include a detailed description of every step of the modeling process (including distribution assumptions, models used, steps, and results). Also include details on the Cox Modeling, including details on the parameter estimates from univariate models, full models, and models after selection of variables.	FDA F2F Meeting on 6/28/2013; EMA F2F Meeting on 7/9/2013	See Sections 4.6 and 5.
60	For the clinical trial sample, use the distribution from the PKDOC database rather than a uniform distribution.	FDA F2F Meeting on 6/28/2013	Completed in new analysis. See Section 5.
61	Detailed description of every step of the Five-Fold Validation process (particularly the order of the steps). Validate that the sequence we used produces the same results as the traditional sequence.	FDA F2F Meeting on 6/28/2013	See Section 4.6 and 5.
62	Evaluate and add 'lowest cut-point' for decision tree and table.	FDA F2F Meeting on 6/28/2013	Completed in new Decision Trees (see Sections 3.3 and 6.5).
63	Address the border values of the cut-points (considering modality precision).	FDA F2F Meeting on 6/28/2013	Cut-points are fixed in the model tables, but different values may be selected by sponsors during trial design based on the precision desired for different modality.
64	More detail on the number of images per subject.	FDA F2F Meeting on 6/28/2013	Please see **Table 13** and **Table 14**.

	Regulatory Recommendation/Comment	**Source**	**Response**
65	Provide the number of endpoints (i.e., 30%, 57%, ESRD) that are contributed from each site registry dataset. Provide event rates if possible.	EMA F2F Meeting on 7/9/2013	See Figure 22.
66	More details on any excluded patients or selection criteria. If possible, provide evidence that this US-only data is representative of the world-wide population, and that there are no significant 'regional' differences. More on the data sources and how they were created.	EMA F2F Meeting on 7/9/2013	A recent publication reports the age of ESRD in 12 European countries to range from age 54 to 60.6 years. This is very similar to what is shown for the PKDOC and US ADPKD populations. Thus, we believe our database is representative of ADPKD patients in Europe and the US. {Spithoven, Orskov 2010}. See also Section 3 (Pages 14 – 19).
67	Establish/prove the link between 30% eGFR decline and clinical relevance. Must address predictive value of 30% decline, in addition to TKVs ability to precisely predict more classical endpoints (57% decline, ESRD, mortality).	EMA F2F Meeting on 7/9/2013	See Section 3.4.6 for additional information.
68	Include the analysis of Baseline eGFR in the final submission package. Provide information to clearly establish that TKV is more predictive during the period when eGFR remains stable and is not deteriorating. Assess TKV 'on top of eGFR.' Using the dataset identified in item 78 (below), perform Joint Modeling and Five-Fold Validations that include Baseline eGFR (with Baseline TKV and Age).	FDA F2F Meeting on 6/28/2013; EMA F2F Meeting on 7/9/2013 FDA follow-up telecon on 9/24/2013	eGFR has been added as a covariate in the new analysis and analyzed with TKV and age. See sections 5 and 6 for results.
69	EMA inquired about the value of a composite endpoint, for example, mortality, transplant, and ESRD. They indicated that, although it is very complex, it may increase the number of events, and there is a standard methodology available for composite endpoints analysis. PKDOC indicated this will be explored.	EMA F2F Meeting on 7/9/2013	By definition, the use of the term ESRD includes both kidney transplant and dialysis. In the analysis, death did not add to the value of the endpoint, likely because transplant and dialysis mitigate the disease effects, and patients receiving this therapy usually die of other causes. Since

	Regulatory Recommendation/Comment	Source	Response
			30% worsening of eGFR, 57% worsening of eGFR, and ESRD are sequential stages of disease progression, they cannot be used as a composite endpoint. For the proposed Context of Use, PKDOC believes that the selected endpoints are most appropriate (see also PKDOC response of 10/2/13).
70	Provide additional Cox modeling to see the effect of other variables (sex, genetics, height, eGFR, proteinuria, weight, etc.)	FDA F2F Meeting on 6/28/2013; EMA F2F Meeting on 7/9/2013	See Section 4.6 and Section 5 for each Endpoint.
71	How did the conversion of data affect the representability of all PKD patients?	EMA F2F Meeting on 7/9/2013	See Section 4.5.1 and **Table 19** (STROBE comparison).
72	The context of use should indicate that TKV is "predictive within the current standard treatment."	EMA F2F Meeting on 7/9/2013	There is no current disease modifying treatment for ADPKD, all therapies are supportive. The Context of Use has been revised based on the addition of eGFR to specify exact usage.
73	What is the median observation for time-in-study?	EMA F2F Meeting on 7/9/2013	See plots in Section 4.2.
74	There is confusion about sex, height, and genotype having an effect. In the briefing book PKDOC states there is an effect. Need to clarify why it appears like there is significance, but in the multivariate analysis it is washed out by TKV.	EMA F2F Meeting on 7/9/2013	See tables and explanations in Sections 5.1, 5.2, and 5.3.
75	Provide arguments why height-adjusted baseline TKV as well as change in TKV over time, are well-suited as biomarkers of enrichment.	EMA F2F Meeting on 7/9/2013	See Results and Conclusions (Sections 5 and 6).
76	Explain how different methods of calculating/estimating eGFR and measuring TKV may impact conclusions.	EMA F2F Meeting on 7/9/2013	See Appendix 8.1: Data Handling for summary of eGFR

	Regulatory Recommendation/Comment	**Source**	**Response**
			calculations.
77	Clarify Documentation a. Add complete explanations, labels, and footnotes to provide clarity for all analysis tables. a. Ensure all acronyms are in the glossary. b. Use Breiman & Spector Notation. c. Include additional explanation for the Median, Lower, and Upper Obs. d. Include additional explanation for the Median Val. e. Include additional explanation for the calculations of PE Median, Lower, and Upper.	FDA F2F Meeting on 6/28/2013; EMA F2F Meeting on 7/9/2013	Changes have been made throughout document.
78	Execute a pre-analysis step using Univariate and Multivariate Cox Modeling on all the analysis datasets (see #1 above). Include these results in the full qualification package. Determine the best dataset to estimate prognostic value of the biomarkers.	Clarifications from final BQRT and SAWP meetings	Completed and shown in Sections 5.1, 5.2, and 5.3.
79	Provide new graphical formats to show the relationship of TKV, eGFR, and age. Do this for both the distribution of TKV, eGFR, and age over the patient population, and for the relative prognostic influence of TKV and eGFR at different combinations of these values.	Clarifications from final BQRT and SAWP meetings	Completed and included in Conclusions (Section 3.3 and 6).
80	Provide more detail on the number of images per subject.	Clarifications from final BQRT and SAWP meetings	Refer to **Table 13** and **Table 14**.
81	In order to further understand the potential for differences when relying on data from the different imaging modalities, please include in your submission the joint modeling work you have already completed (i.e., the work that did not incorporate eGFR in the model) that used the three different imaging-based datasets.	Clarifications from final BQRT and SAWP meetings	All previous modeling work is included in Appendix 8.5.
82	Please include a discussion and any available data results or summaries that aid consideration of the comparability of ultrasound imaging measurements with either CT or MRI methods.	Clarifications from final BQRT and SAWP meetings	See Section 4.3 and referenced publications.
83	As needed, update the context of use, simulation examples and tables, and Results/Conclusion sections.	Clarifications from final BQRT and SAWP meetings	Completed and included in Conclusions (Sections 3.3 and 6).

3.2.3   Questions for Regulatory Agencies

1. **Question:** Do the FDA and EMA agree that the Context of Use clearly describes how TKV will be used by sponsors as a prognostic biomarker to enrich clinical trial population in clinical trials at all stages of ADPKD drug development, including proof of concept, dose-ranging, and confirmatory trials?

   PKDOC Position and Justification: PKDOC believes that the Context of Use as described in Section 3.3 provides clinical trial researchers with a tool to select Baseline TKV, Baseline eGFR, and age cut-off values for use as inclusion criteria in clinical trials. Clinical trial researchers can use the tables supplied to understand how doing so will increase the probability of enrolling patients in the trial who are most likely to progress to a stage of renal disease that will meet the clinical endpoint of interest (See Section 6).

2. **Question:** Do the FDA and EMA agree that the following are clinically relevant endpoints of ADPKD and are adequate to track disease progression?

   a. 30% Worsening of eGFR

   b. 57% Worsening of eGFR (selected based on equivalence to doubling of serum creatinine)

   c. End-Stage Renal Disease

   PKDOC Position and Justification: PKDOC believes that each is a relevant clinical endpoint in a PKD clinical trial, and that TKV can be used as an enrichment biomarker in a trial using any of these as an endpoint. See Sections 3.4.6, 5, and 6.

3. **Question:** Do the FDA and EMA agree that the totality of data accumulated and the scientific evidence generated through the execution of the PKDOC Research plan, is sufficient in supporting the qualification of Baseline TKV, in combination with age and baseline eGFR, as a prognostic biomarker in ADPKD patients?

   PKDOC Position and Justification: PKDOC believes that the rich source of longitudinal data from three academic registries and two observational trials provide both sufficient quantity and diversity of data to support the qualification, and that the modeling and validation approach are state-of-the-art and in agreement with what was previously discussed as an approach to use. The results of the analysis show a strong correlation between baseline TKV and the likelihood of renal disease progressing to one of the three endpoints above, and can reliably be used as an inclusion criterion.

3.3   Context of Use Statement/Statement of Need for and Impact of Proposed Novel Methodology

This document presents evidence to support the regulatory qualification of TKV as a prognostic biomarker for the following Context of Use, based on definitions set forth by FDA's Guidance on the Qualification Process for Drug Development Tools (*Guidance Compliance Regulatory Information*), and the EMA's *"Qualification of novel methodologies for medicine development"*:

- **General Area**: Clinical trial enrichment in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

- **Target Population for Use**: Patients with ADPKD

- **Stage of Drug Development for Use**:   All clinical stages of ADPKD drug development, including proof of concept, dose-ranging, and confirmatory clinical trials.

- **Intended Application:**   Baseline TKV can be applied as a prognostic biomarker that, in combination with patient age and baseline estimated Glomerular Filtration Rate (eGFR), can be used to help identify those ADPKD patients who are at the greatest risk for a substantial decline in renal function defined as (1) 30% worsening of eGFR, (2) 57% worsening of eGFR (equivalent to doubling of serum creatinine), or (3) End-Stage Renal Disease (ESRD, defined as dialysis or transplant).   This biomarker will be used as an inclusion criterion in clinical trials to identify patients likely to show a clinically relevant decline in kidney function during the duration of the trial.   Data are provided showing the calculated risk of each of these outcomes of declining renal function depending on age, total kidney volume, and baseline eGFR.   Tables will be used by clinical trial researchers to determine the inclusion criteria to help select patients who are likely to reach the clinical endpoint of interest within a timeframe practical for the trial.   These criteria include the optimum age, TKV, and eGFR for selecting subjects to be enrolled in the clinical trial.

    TKV can be measured by Magnetic Resonance Imaging (MRI), Computed Tomography (CT) scan, or ultrasound (US) imaging, and the volume calculated by a standard methodology, such as an ellipsoid volume equation (for ultrasound), or by quantitative stereology or boundary tracing (for CT/MRI).

Using the same analysis and modeling approach described in Section 5, PKDOC also examined two other potential biomarkers, the longitudinal change in TKV and the rate of TKV growth. The longitudinal change in TKV did not improve prognostic performance *beyond that provided by baseline TKV and age.* Additionally, the rate of change of TKV requires longitudinal measurements making it an impractical biomarker for use as a clinical trial enrichment criterion.   (See Appendix 8.5 for additional details.) Therefore, these potential biomarkers were not included in this submission.

The following figure and table demonstrate an approach using TKV in drug development to enrich patient population. Figure 5 illustrates how TKV may be used in clinical trials that may be aimed at three different stages of the PKD disease progression (Early Outcome, Disease Progression, and Late Outcome).

Figure 5: Trial Population Enrichment Decision Tree Using TKV as a Prognostic Biomarker
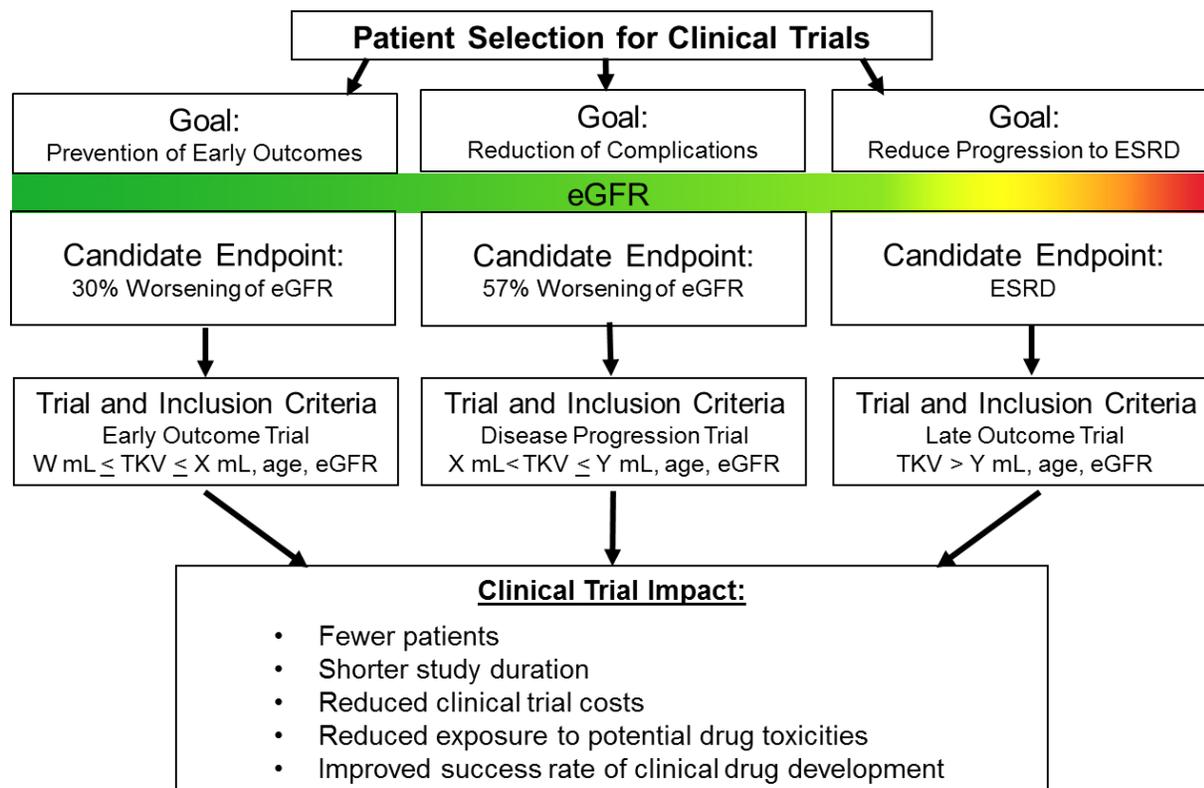
Utilizing the decision tree above, Table 4 demonstrates how the key model components (Baseline TKV, Age, and Baseline eGFR) interact for a trial enrichment example based on the predicted probabilities of a 30% worsening of eGFR according to selected example cut-offs for baseline TKV (< 1 or ≥ 1 liter) and baseline eGFR (≥ 50 or < 50 ml/min/1.73m^2).

Table 4: Example of Trial Enrichment Strategy According to Selected Baseline TKV and Baseline eGFR Cut-Offs for the Predicted Probability of 30% Worsening of eGFR

Follow-Up Times (Years)	Probabilities of Avoiding 30% Worsening of eGFR							
	TKV < 1 L				TKV ≥ 1 L			
	Age: < 40 years		Age: ≥ 40 years		Age: < 40 years		Age: ≥ 40 years	
	eGFR ≥ 50 mL/min	eGFR < 50 mL/min	eGFR ≥ 50 mL/min	eGFR < 50 mL/min	eGFR ≥ 50 mL/min	eGFR < 50 mL/min	eGFR ≥ 50 mL/min	eGFR < 50 mL/min
1	0.991	0.992	0.992	0.991	0.984	0.982	0.985	0.979
2	0.980	0.980	0.981	0.979	0.963	0.959	0.966	0.953
3	0.950	0.949	0.951	0.947	0.907	0.899	0.915	0.884
4	0.917	0.916	0.918	0.913	0.852	0.839	0.863	0.815
5	**0.887**	0.888	0.889	0.884	**0.805**	0.789	0.818	**0.757**

Using the table above, a trial could be designed to include patients who have a 25% probability of reaching 30% reduction in eGFR over five years, by limiting inclusion criteria to TKV >1 L, Age >40 years, and eGFR of <50 mls/min. In contrast, without stipulating inclusion criteria, the probability of reaching 30% reduction in eGFR in five years, would be as low as 11% if patients had a TKV <1 L, Age <40 years, and GFR >50 mls/min. If a trial in younger patients (age < 40 years) with preserved renal function (eGFR >50 mL/min/1.73m2) is desired, a larger baseline TKV (TKV >1 L) would increase the probability of reaching the 30% reduction in 5 years to approximately 20%.

Based on the above probabilities, statistical power calculations may be performed to determine the sample size needed for the endpoint of interest, considering patient characteristics (age, baseline eGFR, and baseline TKV), the study duration, the probability of reaching the endpoint in the control arm, and the hypothetical effect of the therapeutic intervention on the outcomes of interest. These examples also highlight the extreme duration of trials necessary to generate the requisite number of outcome events to be acted upon by the intervention and highlight the desperate need for such biomarker models.

Additional information is available in Section 6.5 – Decision Tree for Use of Baseline TKV and Age for Prognostic Clinical Trial Enrichment.

3.4   Autosomal Dominant Polycystic Kidney Disease (ADPKD)

3.4.1   Prevalence

ADPKD is the most common hereditary kidney disease in the United States. ADPKD is more common than Huntington's disease, hemophilia, cystic fibrosis, sickle cell disease, Down syndrome, and myotonic dystrophy combined {Belibi 2010}. It has a phenotypic prevalence as high as 1:400 individuals when including identification at autopsy {Iglesias 1983, Torres 1985, Wilson 2004, Torres 2009}. Recently, Orphan Status has been granted for tolvaptan in ADPKD by the FDA. By designation of this orphan status for tolvaptan, the FDA's Office of Orphan Products Development acknowledged estimates of diagnosed prevalence to be less than 200,000 individuals in the USA. It has, however, been estimated to be present, although not necessarily manifested, in up to 600,000 individuals in the United States of America, and 12.5 million people worldwide {www.pkdcure.org}. Although its expression during the lifetime varies with the disease's pace, it remains the fourth single leading cause of ESRD in adults and a common indication for dialysis and transplant. The disease accounts for about 8% of all patients on hemodialysis in the United States {Perrone 2001}. ADPKD does not discriminate based on gender, race, ethnicity, or geography. Critically, there is no regulatory-approved treatment to prevent, cure, or delay disease progression, and hence there has been little change in age of development of ESRD over the past decades (Figure 1).

3.4.2   Pathogenesis

There are two genetic forms of ADPKD. ADPKD1 is caused by mutations in the PKD-1 gene (85-90% of cases), and ADPKD2 is caused by mutations in the PKD-2 gene (10-15% of cases) {Hateboer 1999, Wilson 2004}. These two genes encode for the proteins polycystin-1 and polycystin-2 respectively, which are expressed in renal tubular epithelia and other cells. Polycystin-1 is a membrane

mechanoreceptor, which facilitates intracellular responses through phosphorylation and other pathways mediated by polycystin-2, which are not yet not fully defined. Polycystin-2 acts as a calcium-permeable channel and appears to be part of a signaling pathway initiated by polycystin-1. Both types of ADPKD present with similar pathologic and clinical features, but ADPKD2 has a later onset of symptoms. ADPKD2 patients also have fewer cysts and smaller TKV than ADPKD1 patients at any given age. It has been speculated that cysts are initiated at a later age in ADPKD2, but once initiated, expand at a similar rate {Harris 2006}. There are also a small number of patients with ADPKD with no demonstrable genetic mutations, suggesting other genetic mechanisms as yet unidentified {Wilson 2004}.

In ADPKD, each renal tubular epithelial cell carries a germ-line mutation. These cells are hypothesized to be protected by the normal PKD-1 or PKD-2 allele inherited from the parent without ADPKD. When this allele is inactivated by a somatic event (mutation or otherwise) within a solitary renal tubular epithelial cell, this triggers a complex array of molecular processes leading to enhanced cell proliferation and abnormal cell-cell and cell-matrix interactions as well as changing from a reabsorptive to a secretory phenotype. In ADPKD patients, a phenotypic conversion within a tubule epithelial cell commits it to the formation of a cyst rather than an elongating tubule {Grantham 2011b}. A cyst is formed in a renal tubule when focal epithelial cell proliferation provokes radial expansion forming a sac-like protrusion out of the tubule segment. The saccular cyst fills with fluid from glomerular ultrafiltrate that enters from the afferent tubule segment. As the tubular epithelial cells continue to proliferate, the progressively expanding cysts enlarge and eventually separate from the parent tubule to become isolated sacs filled with fluid. It has been demonstrated that 70% of cysts do not communicate with the nephron {Grantham 1987}. Fluid then is secreted into the cyst cavity in response to the cyclic-AMP-dependent transport of chloride and water into the lumen. Cellular proliferation and fluid secretion may be accelerated by cyclic adenosine monophosphate (cAMP), growth factors such as epidermal growth factor (EGF), adenosine triphosphate (ATP), cytokines, and lipid factors. Arginine vasopressin (AVP), through cyclic AMP, leads to chloride secretion into the cysts and promotes increased proliferation of the lining cells. Since humans are terrestrial, hydropenic animals, AVP is continuously elaborated except when large amounts of fluid may be drunk over a short interval. Secretion of AVP has been found to be excessive in ADPKD {Boertien 2012, Meijer 2010}, in part due to impaired urinary concentrating ability of ADPKD kidneys {Zittema 2012}. AVP, through activation of cAMP, promotes cellular proliferation and fluid secretion by cysts and is a dominating factor that controls the rate of cyst and kidney enlargement in patients with ADPKD {Grantham 2011b}.

Cysts within glomerular capsules, proximal tubules, and loops of Henle are seen at the earliest stages for ADPKD, but during the later stages, these cysts diminish in abundance and cysts in the collecting ducts supervene {Grantham 2011b}. Morphologic features that distinguish principal collecting cells from intercalated collecting duct cells are often lost as the patient ages and the cysts expand. The epithelium within the cysts generates chemokines, cytokines, angiogenic factors, interstitial collagens, and other matrix proteins in a failed attempt at injury repair {Grantham 2011b}.

Hundreds to thousands of renal cysts develop and grow over time, some as large as 10-20 cm in diameter. As previously noted, many cysts form *in-utero* and to some extent throughout life, although the relative magnitudes of pre- and post-natal formation remain to be determined. Preliminary evidence in humans and animal models of the disease suggests that cysts formed *in-utero* might grow faster than those that form in adults, and are likely to dominate the landscape of renal cysts observed by clinicians by

ultrasonography, CT, and MRI in both children and adults {Grantham 2006a}. The severity of ADPKD is related to the number of times and the frequency with which the cystogenic process occurs within the kidneys over the life of the patient {Grantham 2006b} and the rate of growth of existing cysts. Epidemiological studies suggest that fewer than half of those with the disease are diagnosed during their life, with a majority of diagnoses occurring on autopsy after death from other causes {Iglesias 1983}. Although not specifically evaluated in those studies, these individuals may have had milder manifestations of disease. It is possible that many of these individuals may have had PKD2, which is generally a milder disease with later age of ESRD. Indeed, surveys of general populations reveal a PKD2 prevalence of 30%, in contrast to the 10-15% prevalence noted in diagnosed ADPKD populations {Harris 2006}.

The fundamental processes of cellular proliferation and apoptosis are disturbed in ADPKD. Apoptosis is abnormally persistent and destroys much of the normal renal parenchyma {Wilson 2004}. The expanding fluid-filled masses elicit secondary and tertiary changes within the renal interstitium resulting in thickening and lamination of the tubule basement membranes, infiltration of macrophages, and neovascularization. Fibrosis within the interstitium begins early in the course of the disease {Grantham 2006b}.

Physical disruption of the renal parenchyma by cysts has been commonly advanced as an explanation for the renal insufficiency that eventually develops in most patients. An inverse relationship has been observed between kidney volume and the capacity to concentrate the urine {Gabow 1989}. Similar observations have been made in children with ADPKD {Seeman 2004}. The potential for cysts that develop in medullary collecting ducts to affect the function of hundreds of upstream nephrons has been postulated as a mechanism for kidney failure in ADPKD {Grantham 2012}.

There is increasing evidence that ADPKD patients experience kidney damage long before a change in iothalamate clearance or eGFR can be reliably detected {Grantham 2011b, Meijer 2010}. Hypertension is detected in some children before significant increases in renal volume can be reliably measured {Cadnapaphornchai 2009, Seeman 2003}, consistent with the view that injury may be caused by innumerable kidney cysts too small to be detected by US, CT, and MRI {Seeman 2003}. Renal blood flow is decreased and renal vascular resistance is increased in ADPKD before changes in GFR can be detected {Chapman 2012, Meijer 2010, Torres 2007}. Moreover, the hyperfiltration documented early in the course of ADPKD {Wong 2004} suggests that radical readjustments in renal hemodynamics may occur as the cysts disturb the delicate anatomy of the cortex, especially the medulla. Therefore, kidney damage with loss of functioning glomeruli may occur very early in the course of the disease. Hypertension, hyperfiltration, and impaired maximal urine concentration likely result from the impact of these cysts {Grantham 2012}.

Cyst expansion within the kidney invariably forces anatomic accommodation by adjacent tubules, vasculature, and interstitium. The distending pressure in the cyst mass is higher than in adjacent tubules, causing partial or complete obstruction. Lumen compression by cysts slows fluid flow through tubules, blood vessels, and lymphatics. Medullary cysts can have a more serious overall potential effect than cortical cysts because of the increased potential impact on many more upstream tubules. In addition, the effect on overall renal blood flow and urine formation is further magnified in patients who develop greater numbers of cysts {Grantham 2011a}.

The distortion of intrarenal arteries and arterioles and the obstructed urine flow of renal tubules both contribute to the increased production of intrarenal renin that activates angiotensin II, a vasoconstrictor. Reduced renal blood flow seems to precede the decline in GFR by several years, with resultant regional hypoxia and cellular injury, and is decreased in hypertensive ADPKD individuals prior to loss of renal function, resulting in an increased filtration fraction {Grantham 2011b, Schrier 2009}. There are many similarities in the pathogenesis of the interstitial inflammation and fibrosis seen in ADPKD and the renal response to obstructive uropathy {Grantham 2011b}. In both conditions, renal epithelial cells generate chemokines and cytokines; in cystic disease these biologically active compounds accumulate in the cyst fluids to high levels {Chevalier 2000}. Mononuclear cells, including macrophages and fibroblasts, invade the renal interstitium to create a low-grade tubule-interstitial reaction, and there is massive apoptosis causing the disappearance of normal parenchyma.

As cysts enlarge, these disruptive processes are repeated endlessly, renal parenchymal integrity is further compromised, and the efficiency of the compensation for a reduced GFR decreases. Non-cystic nephrons are injured, undergo apoptosis, and disappear, leaving extensive replacement cysts held in place by thick bands of fibrotic material and a much-diminished amount of functioning parenchyma. In patients with moderate or far-advanced disease, renal arterioles exhibit intimal thickening, smooth muscle hypertrophy, and global, but not focal, glomerular sclerosis {Zeier 1992}. Tissue ischemia further activates the local production of angiotensin II, contributing again to renal injury.

Renal insufficiency in patients with ADPKD is primarily the consequence of cyst formation and expansion. The mass effect of expanding cysts slows and blocks the flow of urine in non-cystic tubules and disrupts delicate vascular relationships in the cortex and medulla, leading to secondary interstitial inflammation and fibrosis.

TKV directly reflects the number of cysts and their size. The rate of cyst expansion determines the rate of kidney enlargement in patients with ADPKD. TKV is a direct measure of the underlying pathogenic process in ADPKD.

3.4.3   Natural History

Data addressing the rate of decline of GFR in ADPKD are summarized in **Table 5**. Progression rates vary from 2.7 to 6.5 ml/min/1.73 m^2 with slower rates of GFR decline in those with initially well-preserved kidney function.

Table 5: Rate of Decline of GFR in ADPKD

Initial GFR (ml/min/1.73 m^2)	Rate of decline (ml/min/1.73 m^2/yr$\pm$SE)	Method	N	Intervention
25-55	5.9 $\pm$ 0.3	125I-iothalamate clearance	141	Enhanced BP control and dietary protein restriction {Klahr 1995}
13-24	4.4 $\pm$ 0.2	125I-iothalamate clearance	59	Enhanced BP control and dietary protein restriction {Klahr 1995}
30-50	5.8 $\pm$ 0.2	Creatinine clearance	109	None. Favored slow

50-60	5.3 $\pm$ 0.4		48	progressors due to requirement for 4-yr f/u {Choukroun 1995}
73 $\pm$ 21 (SD) M 71 $\pm$ 23 (SD) F	2.99 1.98	Estimated using MDRD equation.	84 M 145 F	None {Fick-Brosnahan 2002}
83 $\pm$ 5 (SE) 77 $\pm$ 6 (SE)	2.8 4.2	Creatinine clearance	12 12	BP control with amlodipine BP control with enalapril {Ecder 2000}
91.4 $\pm$ 5.4	2.8 $\pm$ 0.9	125I-iothalamate clearance; Cockroft-Gault estimate	9	None {King 2000}
74 83	5.3 2.7	Creatinine clearance	14 19	Diuretics ACE inhibitors {Ecder 2001a}
>80	5.8 $\pm$ 1.3	Cockroft-Gault estimate	30	None {Gonzalo 1996}
82	4.3 $\pm$ 4.2	Cockroft-Gault estimate	72	None {Ecder 2001c)
>80 <80	3.8 $\pm$ 3.5 6.5$\pm$ 4.6	125I-iothalamate clearance; non-progressors excluded	45 65	None (A. Chapman, personal communication)
112 $\pm$ 3 89 $\pm$ 2	2.7 $\pm$ 0.7 4 $\pm$ 0.7	Inulin clearance	61 28	Normotensive (placebo and enalapril had same rate of GFR decrease. Hypertensive {van Dijk 2003}
73.8 $\pm$ 22	No change over 6 months	Measured GFR (iohexol plasma clearance	15	Sirolimus vs. conventional therapy for 6 months with crossover {Perico 2010}
57.9 $\pm$ 22.4	No change over 6 months	Measured GFR (iohexol plasma clearance	12	Somatostatin vs. conventional therapy for 6 months with crossover{Ruggenenti 2005}

Note: normal volume of two adult kidneys is less than 300 cc

There are very limited data addressing the rate of decline of GFR in the early stages of ADPKD progression. Longitudinal observations of children revealed no progression in the vast majority before age 25 {Fick-Brosnahan 2001}. The relationship between clinical symptoms of ADPKD and GFR is highly variable likely due to the striking dissociation between expansion of TKV and GFR early in the course of disease. This concept was originally proposed by Franz and Reubi who performed serial measurements of GFR in subjects with ADPKD and found slower progression rates in the early stages (i.e., at younger age) of ADPKD {Franz 1983}.

ADPKD is notable for a long period of apparently stable kidney function as measured by GFR despite the relentless expansion of TKV due to growth of cysts and the resultant compression of normal renal architecture and vasculature, interstitial fibrosis, tubular atrophy, and progressive kidney dysfunction

{Zeier 1992}. GFR remains at or near normal until kidneys are markedly enlarged {Grantham 2006b}. Stability of GFR results from hyperfiltration of surviving nephrons. The finding of stable GFR when ADPKD kidneys are dramatically enlarged, distorted by multiple cysts and fibrotic tissue provides false reassurance as to stability of disease progression. Once serum creatinine levels begin to rise in these patients, GFR falls at a faster and more uniform rate than in other types of progressive renal disease {Klahr 1995}.

Currently, changes in GFR (frequently estimated from changes in serum creatinine) are considered the gold standard for quantifying the progression rate in most chronic renal diseases {Levey 2009}. However, given that these changes are seen only once the kidney architecture has been grossly and irreversibly distorted, GFR may not be suitable to predict the likelihood of progression at early stages of the disease {Wuthrich 2009}. ADPKD progresses slowly over decades, as the size of the kidneys increase from a normal range (150-200 cm^3) in adolescence to >1,500 cm^3/kidney later in life {Wuthrich 2009}. For PKD1, the more aggressive form of the disease affecting 85%-90% of cases {Chapman 2009, Hateboer 1999}, ESRD develops in approximately 50% of affected persons by age 53 years {Parfrey 1990; Churchill 1984}.

However, renal complications associated with the development and enlargement of kidney cysts arise long before renal function begins to diminish. Many patients diagnosed with ADPKD will experience one or more severe symptoms attributed to the enlargement of their kidneys. All of the common signs and symptoms related to progression of ADPKD are associated with increased TKV including hypertension, chronic pain or heaviness in the flank or abdomen, and hematuria and cyst hemorrhage {Grantham 2006b}.

Pain with or without hemorrhage is the most frequent symptom reported by both adults and children with ADPKD and is associated with increased renal size. Currently active pain occurs in approximately 50-60% of all ADPKD individuals {Gabow 1990b}. Pain may also be caused by renal hemorrhage, the passage of renal stones, infected cysts, and pyelonephritis {Grantham 2006b}.

Pain management is initially conservative but can be challenging with many patients unresponsive to analgesic treatment. When one or more cysts can be identified as causing pain, specific surgical intervention may be required. However, in about half of these patients, the specific cyst causing pain cannot be identified {Jouret 2012}. In these cases, the indiscriminate excision of several cysts has produced symptomatic relief. However, not every cyst can be removed and, over time, residual cysts continue to enlarge with the return of associated symptoms {Grantham 2006b}.

Some patients with the most severe pain are unresponsive to both surgery and narcotics. Reports of nephrectomy to manage symptoms in some patients are available. However, this is a major undertaking in ADPKD patients and is associated with significant morbidity and loss of functioning kidney parenchyma and kidney function, especially when conducted pre-transplant {Kirkman 2011}.

Polycystic kidneys are particularly susceptible to traumatic injury, with hemorrhage occurring in approximately 60% of patients. Even mild trauma can result in intrarenal or retroperitoneal bleeding with intense pain that often requires narcotics for relief {Levine 1987}.

Cysts are associated with excessive angiogenesis and many patients have been documented to have had intracystic bleeding {Levine 1985}. This can cause rapid expansion of the cyst, again associated with

intense pain but without evidence of hematuria. In certain cases of cystic bleeding, the cyst can rupture into the collecting system with resultant gross hematuria. It can also rupture into the subcapsular compartment and eventually dissect through the renal capsule filling the retroperitoneal space. In cases of massive bleeding, subcutaneous ecchymoses can result. Over 50% of PKD patients experience renal hemorrhage caused by cysts by the age of 30 and occur at any age, severely diminishing their quality of life {Grantham 2006b}.

Cyst expansion also results in intrarenal ischemia which activates the renin-angiotensin-aldosterone system (RAAS) contributing to the development and maintenance of hypertension. This is an early and frequent finding of ADPKD occurring in approximately 60% of patients before their renal function has become impaired. The average age of hypertension, although highly variable, is approximately 30 years {Grantham 2006b}. Hypertension in ADPKD is not limited to adult patients {Ecder 2001b} and has been demonstrated in 22% of children with ADPKD at the time of diagnosis {Sedman 1987}. Another study documented an 18% incidence of hypertension in children {Fick 1994}. Importantly, hypertensive children and hypertensive adult men and women ADPKD patients with normal renal function demonstrate significantly greater TKV than their normotensive counterparts {Gabow 1989; Fick-Brosnahan 2001, Chapman 2003}. Hypertension has a significant impact on morbidity and mortality in ADPKD. Those patients with hypertension have a more rapid loss of renal function, a higher risk for progression to ESRD, and are more at risk for cardiovascular disease and death, the most frequent cause of mortality in ADPKD patients. The risk for development of hypertension in ADPKD in relation to TKV is now quantified with a 1.47 increased risk associated with every 100 ml increase in TKV {Chapman 2012}. Increased cardiovascular risk may be accentuated by hypertriglyceridemia, which was noted in just less than half of fasting pediatric patients, and hypercholesterolemia, which was noted in nearly one-fifth of this study group {Tee 2004}.

Left ventricular hypertrophy (LVH) has also been reported to be prevalent in patients with ADPKD. One study reported a 48% prevalence of LVH in hypertensive ADPKD patients. In this study, there was a significantly higher frequency of LVH in ADPKD men (46 vs. 20%) and women (37 vs. 12%) compared with healthy control subjects. Additionally, LVH was detected even in 23% of normotensive ADPKD patients {Chapman 1997}. There is also a significant correlation between hypertension and left ventricular mass index (LVMI) both in children and adults with ADPKD {Ivy 1995, Chapman 1997}. This relationship between systolic blood pressure and LVMI in children with ADPKD was not observed in unaffected siblings. A recent study using cardiac MRI in younger (~36 years of age) patients with a relatively short duration of hypertension and eGFR>60 ml/min/1.73m^2 demonstrated a low prevalence of LVH, possibly related to early and more frequent use of angiotensin blockade in these patients {Perrone 2011}.

ADPKD patients are at increased risk of cardiac valve defects. They also have been shown to have a relative risk of 5.6 (95% CI: 2.7-7.3) for intracranial aneurysms compared to the general population, and higher than for patients with atherosclerosis {Rinkel 1998, Vlak 2011}. There have been anecdotal reports of dissecting abdominal aortic aneurysms {Nacasch 2010}; however, these have not been confirmed to occur in increasing prevalence in other observational cohorts {Torra 1996}.

Additional extrarenal manifestations include liver, thyroid, pineal, subarachnoid, and epididymal cysts and diverticular disease {Chapman 2009}.

Enlarged kidneys can also be disfiguring. Increasing volume results in deformation of the abdomen, increases belt and dress sizes substantially, and causes pain with seat belts. The additional mass in the abdomen affects posture during standing and walking, which contributes to lower back pain. Large kidneys can also affect diaphragmatic motion and disturb sleep. PKD clinicians report that patients find the enlargement of the abdomen very stressful {Grantham 2006b}. Surveys of ADPKD patients obtained using the methodology from FDA's Guidance for the Development of Patient Reported Outcomes reveal great concern about abdominal discomfort and pain {Cole 2011}.

A significant number of clinical reports strongly support the view that the onset of abdominal pain, hypertension, gross and microscopic hematuria, and renal insufficiency are the result of progressive enlargement of the cysts. Remarkable structural disease progression occurs during the prolonged early phase of the disease prior to deterioration in GFR. Thus, tools to measure TKV and the rate of its progression allowing to forecast changes in both kidney structure and function are critical for an accurate assessment of renal prognosis {Chapman 2009; Bae 2010}. Therefore, measuring kidney size with accurate and sensitive methodologies and forecasting changes in kidney structure and function has merit as a strategy to assess how serious the potential constellation of secondary renal complications might be in individual patient {Grantham 2006b}. As ADPKD progresses by increases in the number and size of renal cysts in accordance with increasing TKV, PKDOC proposes that TKV is an excellent marker of disease progression in this disorder.

3.4.4   Diagnosis

The diagnosis of ADPKD usually relies on diagnostic imaging. Renal ultrasound (US) is commonly used due to its high reliability and because of cost and safety. The original criteria developed for at risk individuals in PKD1 families required for age 18-30, at least two cysts in either or both kidneys, for age 30-59, at least two cysts in each kidney, and for age 60 or older, at least four cysts per kidney {Ravine 1994}. Revised criteria have been proposed to improve the diagnostic performance of sonography for ADPKD in individuals from families of unknown genotype {Pei 2009} as the original criteria were sufficiently sensitive only in PKD1. The presence of at least three (unilateral or bilateral) renal cysts is sufficient for diagnosis of at-risk individuals aged 15–39 years, whereas two or more cysts in each kidney are required for ages 40–59 years. For at-risk individuals aged ≥60 years, four or more cysts in each kidney are required. The requirement of three or more cysts (unilateral or bilateral) has a positive predictive value of 100% in the younger age group and minimizes false-positive diagnoses, as 2.1 and 0.7% of truly unaffected individuals or the general population, younger than 30 years, have one and two renal cysts, respectively. In 30–39 year olds, both the original and the revised criteria have a positive predictive value of 100%. Although the specificity and positive predictive value of the sonographic criteria are very high, their sensitivity and negative predictive value when applied to PKD2 in the 15–29 (69.5 and 78%, respectively), 30–39 (94.9 and 95.4%, respectively), and 40–59 (88.8 and 92.3%, respectively) years old groups are lower.

A more recent study has addressed the prevalence of kidney cysts in 1948 healthy individuals being considered as potential kidney donors {Rule 2012}. The 97.5[th] percentile for number of cortical and medullary cysts >5 mm increased with age: one for men and one for women in the 18- to 29-year group; two for men and two for women in the 30- to 39-year group; three for men and two for women in the 40- to 49-year group; five for men and three for women in the 50- to 59-year group; and 10 for men and 4 for

women in the 60- to 69-year group. It is important to note that the vast majority of these individuals have cyst diameters less than 1 cm, a size typically seen in most ADPKD individuals.

There are limitations to genetic testing, either by linkage or mutation analysis. Linkage analysis requires accurate diagnosis, availability, and willingness of a sufficient number of affected family members (at least three) to be tested, and is feasible in fewer than 50% of families. *De novo* mutations can also complicate interpretation of results which can occur in up to 10% of ADPKD individuals. Molecular testing by direct DNA sequencing is now possible with likely mutations identified in 85-90% of patients {Rosetti 2012}. However, as most mutations are unique and up to one-third of PKD1 changes are missense, the pathogenicity of some changes is difficult to prove {Torres 2009}.

3.4.5   Current Standard of Care

At present there are no approved therapies specifically targeting ADPKD, and as such, there are no disease-specific modifying interventions currently available. Current patient management aims to ameliorate the symptoms of ADPKD and the complications of hypertension and reduced eGFR associated with this disorder. The most common complications of ADPKD arise from the kidney due to cyst burden and include hypertension, pain, gross hematuria, urinary tract infections, and kidney stones. All of these complications are associated with increases in kidney size or TKV. Management strategies include control of hypertension and use of analgesics in addition to treatment of the causes of pain, as well as strategies to reduce the occurrence of kidney stones and urinary tract infections and the duration of gross hematuria {Schrier 2006, Masoumi 2008}.

Hypertension

Hypertension is one of the most frequent complications among ADPKD patients. The early onset of hypertension is close to 80% even before a substantial decline in kidney function has occurred. Frequently, this may lead to hypertension remaining undiagnosed and untreated for several years {Schrier 2006}. Untreated hypertension predisposes to development of left ventricular hypertrophy (LVH) with increased cardiovascular mortality risk {Fick 1995}. Hence, frequent BP monitoring and early initiation of anti-hypertensive therapy in ADPKD patients remains the most effective management strategy to date {Schrier 2006}. Activation of the renin-angiotensin- aldosterone system (RAAS) occurs early in ADPKD {Ecder 2001b}. Moreover, the use of angiotensin-converting enzyme inhibitors (ACEI) has been shown to be effective at decreasing left ventricular mass index in ADPKD {Ecder 1999}, especially when rigorous BP control (<120/80 mmHg) {Schrier 2002} is maintained. A slowing of the annual decrease in GFR in ADPKD patients treated with ACEI has also been reported {Ecder 2001a}. Similar beneficial effects of ACEI therapy on prevention of an increase in albumin/creatinine in ADPKD patients have been noted {Ecder 2000}. Epidemiologic studies reveal that age of onset of renal failures has been extended in patient cohorts from Colorado and Denmark in association with use of inhibitors of the renin-angiotensin system in addition to lower blood pressure levels and improved blood pressure control. However, definitive results related to the effectiveness of RAAS blockade on slowing progression of renal and cardiac disease in ADPKD must await the outcome of the prospective randomized HALT PKD clinical trial (scheduled to conclude 6/30/2014){Chapman 2010}.

Pain

Pain is the most frequent complaint among ADPKD patients, occurring in approximately 60% of affected individuals {Gabow 1990b}. The occurrence of severe abdominal or flank pain may necessitate therapeutic intervention. Pain may occur on either an acute or chronic basis related to different etiologies in ADPKD.

Acute Pain

Acute pain may arise due to cyst rupture and hemorrhage, cyst infection, renal stone, or, most commonly, due to cystitis {Masoumi 2008}. In these cases, differential diagnosis of the cause of the pain and targeted therapy to alleviate the underlying problem is the standard of care best employed. Treatment of cystitis is the same as for the general population. Cyst infection, which may present with symptoms similar to pyelonephritis, including low-grade fever and isolated flank pain, is treated with lipophilic antibiotics such as ciprofloxacin, chloramphenicol, vancomycin, or trimethoprim-sulfamethoxazole, which demonstrate better penetration into the cyst cavities {Masoumi 2008}. If treatment is ineffective, cyst drainage may be necessary and typically occurs in cysts greater than 5 cm in diameter. Cyst rupture in ADPKD may present with acute colicky pain and gross hematuria. However, gross hematuria may also be associated with sports injury or kidney stone. Usually episodes of gross hematuria are limited, rarely necessitating transfusion, nephrectomy, or renal arterial embolization. Typically bed rest, hydration, and local compression are sufficient to make these episodes self-limiting. Use of non-steroidal anti-inflammatory drugs (NSAIDs) should be limited when gross hematuria is present. Flank pain, hematuria, and urinary tract infection are also symptoms of nephrolithiasis {Masoumi 2008}. Uric acid stones occur more frequently in ADPKD compared to the general population, occurring in approximately 20% {Levine 1992}. Treatment may include short-term administration of NSAIDs to alleviate immediate renal colic symptoms, reduce inflammation and edema thereby facilitating stone passage {Masoumi 2008}. Extracorporeal shock wave lithotripsy or nephrolithotomy are beneficial in patients with renal calculi. For long-term benefit, increased fluid intake, greater than three liters of fluid a day, helps to reduce the rate of stone formation.

Chronic Pain

Larger kidney volumes and increased hepatic cyst volume are associated with dull to severe chronic pain. This may be present as mechanical back pain resultant from hypertrophic lumbosacral muscle groups or pain due to traction of the renal capsule due to enlarging cysts. Conservative interventions including physical therapy measures and/or use of systemic analgesics are the primary treatment. Severe pain may require more invasive measures to achieve control, including transcutaneous nerve stimulation, autonomic plexus blockade, neuromodulation by spinal cord stimulation or neuro-axial opioids, and local anesthetics. In severe cases, surgical decompression of large cysts or nephrectomy may be necessitated.

Liver Cysts

Hepatic cysts are the most common extrarenal manifestation of ADPKD occurring in over 85% by age 30, and hepatomegaly is a frequent complication. Similar to acute renal complications, hepatic cyst infection and/or rupture may occur, although this is infrequent. Antibiotic therapy with percutaneous cyst drainage under ultrasound or CT guidance is useful for treatment. Massively enlarged liver cysts may displace adjacent organs with resultant early satiety and pain. Cyst fenestration and liver resection-

fenestration are surgical strategies that may be employed when malnutrition and/or intolerable pain are present {Masoumi 2008}.

Intracranial Aneurysms

Patients with ADPKD are at an increased risk for intracranial aneurysm compared to the general population. Because familial clustering of ruptured intracranial aneurysm (RICA) occurs, a positive family history of RICA or unruptured ICA is an indication for screening, preferably by magnetic resonance angiography (MRA), after age 20 {Schrier 2006, Masoumi 2008}. Based on a negative screening by MRA, a 10-year follow-up screening is only indicated in those subjects with a positive family history of RICA. Treatment of larger aneurysms >10 mm includes clipping or endovascular occlusion by coil. The majority of intact small aneurysms do not increase in size over time, and small asymptomatic aneurysms < 5 mm may be managed with imaging every two to five years.

Summary

Based on current knowledge, the optimal standard of care {Schrier 2006, Masoumi 2008} for ADPKD patients includes:

- Early diagnosis and treatment of hypertension with target for blood pressure <130/80 mm Hg

- Initiation of RAAS inhibitor therapy in patients with blood pressure >130/80 mm Hg

- Early diagnosis and treatment of urinary tract infection, cyst infection, or nephrolithiasis

- Pain management that includes diagnosis and treatment of the cause, where possible, and adoption of the most conservative effective measure for management of chronic pain

- Heart-healthy, salt-restricted diet

- Management of the complications of reduced GFR (anemia, osteodystrophy, electrolyte disturbance)

- Renal Replacement Therapy

3.4.6   Selection of Endpoints Used to Derive the Predictive Models

The following clinically relevant ADPKD endpoints were modeled:

- 30% Worsening of eGFR: Evidence to support the utility of a 30% decline in eGFR as a predictor for the future development of kidney failure has been assembled by the CKD Prognosis Consortium and discussed extensively at a combined FDA/NKF conference held in Baltimore, MD during December, 2012. The final conference results have been submitted for publication (personal communication, AS Levey) and the results have been published in abstract form {Coresh 2013}. Analyses were conducted on 21 cohorts consisting of 722,221 participants of whom 7,529 reached ESRD during an average follow-up of 2.4 years. A 30% decline in eGFR was associated with a five- to six-fold increase in the hazard of developing ESRD compared to no decline, irrespective of whether baseline eGFR was above or below 60.

- <u>57% Worsening of eGFR (selected based on equivalence to doubling of serum creatinine)</u>: Doubling of serum creatinine has been accepted by regulatory agencies as an endpoint predictive of the development of ESRD {Levey, 2009} The CKD prognosis consortium also addressed the impact of larger declines in eGFR on predicting ESRD. A 57% decrease in eGFR was associated with a 31.1-fold increase in the hazard of developing ESRD compared to no decline {Coresh 2013}.

- End-Stage Renal Disease defined as dialysis or kidney transplant.

Mortality and new onset hypertension or uncontrolled hypertension were also examined as possible endpoints for which TKV could be predictive. The analysis showed that age is the primary predictor of mortality; TKV is not a good predictor of mortality. This is likely the result of successful interventions in the course of the disease, namely dialysis and kidney transplantation. The relationship between TKV and new onset hypertension or uncontrolled hypertension was weak largely because 85% of the patients were hypertensive prior to the first measurements of TKV and were not eligible to be assessed for new onset of hypertension. For CKD Transition from Stage 1-2 to Stage 3 or higher, baseline age and TKV were not statistically significant in the joint model. Since patients with CKD 1 and 2 were mainly young patients with low baseline TKV values, a significant association for the probability of a transition from CKD 1-2 to 3 and above could not be demonstrated. This is most probably due to loss of power when we look at categorical endpoint definition (CKD stages) as opposed to a quantitative one such as eGFR. Previous analysis on CKD transitions has shown that the slope of TKV growth was a better prognostic than TKV at baseline. However, since determining a slope would require two TKV measurements separated by at least six months, it was deemed impractical for use as a clinical trial inclusion criterion.

Gross hematuria, kidney stones, severe urinary tract infection (defined as pyelonephritis or cyst infection), hospitalization for PKD-related complications, pain, abdominal distension, and abdominal fullness are also important clinical features of ADPKD. However, validated tools to accurately capture these symptoms were not available at the time of data collection in the long-term registries; thus, we are unable to assess TKV as a predictor of these outcomes in the retrospective data set. Efforts to design and validate a patient reported outcomes tool in ADPKD are in progress {Cole 2011}, and this tool may permit collection of these data for inclusion in the ADPKD database in the future.

3.5   Animal Models

While rodent models of PKD do not entirely recapitulate the human phenotype, kidney enlargement in these animal models consistently precedes the development of renal insufficiency. Abundant evidence for renal functional deterioration in association with enlargement of TKV has been shown in multiple animal models {Grantham 2006b, Torres 2008}.

Numerous rodent genetic models of polycystic kidney disease are currently available, and the use of TKV as a prognostic biomarker (or biomarker of disease progression) is supported in these models. These have arisen through spontaneous mutations, or by random mutagenesis, transgenic technologies, or gene-specific targeting. They share common pathogenic features with human PKD, including increased epithelial cell proliferation and transepithelial fluid transport, and have contributed to the understanding of the underlying pathophysiology of PKD {Guay-Woodford 2003}.

Table 6 summarizes the models originating from spontaneous mutations or through chemical or insertional mutagenesis {Torres 2007}. The majority of these models have an autosomal recessive inheritance. Some models have phenotypes resembling ARPKD (cpk, bpk, and orpk mice), whereas others resemble ADPKD (pcy and jck mice, PCK and LPK rats). The distal nephron and collecting duct are involved in most of these models, whereas the cystic disease in the Han:SPRD rat affects mostly the proximal tubules.

Table 6: Rodent Models of PKD Originating from Spontaneous Mutations or through Chemical or Insertional Mutagenesis {Torres 2007}

Model	Inheritance	Renal Path-ology	Progression	Extrarenal pathology	Gene	Protein	Human disease
Mouse							
Cpk	AR[a]	PT→CD	Rapid	BD[b], P[b]	Cys1	Cystin	?
Bpk	AR	PT→CD	Rapid	BD	Bicc1	Bicaudal C	?
Jcpk	AD/AR	GI/all tubules	Slow/rapid	BD	Bicc1	Bicaudal C	?
Orpk	AR	PT→CD	Rapid	BD, PD	TgN737	Polaris	?
inv	AR	PT→CD	Rapid	BA, P, SI	Invs	inv	NPHP2
pcy	AR	CD, nephron	Slow	ICA	Nphp3	Nephrocystin-3	NPHP3
jck	AR	CD, DT, LH	Slow	-	Nek8	Nek8	NPHP9
kat, kat2J	AR	GI, PT	Slow	FD, MS, HC, An	Nek1	Nek1	SRPS
Rat							
Han:SPRD	AD/AR	PT	Slow	L[c]	Pkdr1	SamCystin	?
wpk	AR	PT→CD	Rapid	HC	Mks3	Meckelin	MGS
pck	AR	CD, DN	Slow	BD	Pkhd1	Fibrocystin	ARPKD
LPK	AR	CD	Slow	LVH	Nek8	Nek8	NPHP9

[a]Focal dilatation of bile ducts in old heterozygotes. [b]In DBA/2J background. [c]Liver cysts in old females. AR, autosomal recessive; AD, autosomal dominant; PT, proximal tubule; CD, collecting duct; GI, glomeruli; C, cortex; OM, outer medulla; DN, distal nephron; BD, biliary dysgenesis; P, pancreatic cysts or fibrosis; PD, polydactyly; BA, biliary atresia; SI, *situs inversus*; ICA, intracranial aneurysm; FD, facial dysmorphism; MS, male sterility; HC, hydrocephalus; An, anemia.

Many gene-targeted knockouts of mouse PKD1 and PKD2 result in similar phenotypes, as shown in **Table 7**. In homozygotes, kidney development proceeds normally until embryonic day 15.5, at which time point cystic dilatation of renal tubules and cystic degeneration in the pancreas become evident. All homozygous fetal mice develop polyhydramnios and hydrops fetalis resulting in embryonic or perinatal death. Defects in axial skeletal development and laterality defects have been described in PKD1- and PKD2-targeted mice, respectively. On the other hand, mice with a heterozygous mutation of PKD1 or PKD2 only develop scattered renal and hepatic cysts late in life. Because of their clinical course, neither homozygous nor heterozygous PKD1 or PKD2 knockouts are adequate to test potential therapies for

ADPKD, although a few studies have used treated pregnant mice to study the effect of therapeutic interventions on the homozygous embryos and of heterozygous mice despite their very mild phenotype {Torres 2007}.

To overcome the limitations of the constitutive knockouts, critical sequences of PKD1 or PKD2 have been flanked with loxP sites (specific 34-bp-long DNA sequences) and conditionally removed by the expression of Cre recombinase (a protein that catalyzes recombination between loxP sites). Expression of Cre recombinase is placed under the control of a site specific promoter (e.g., kidney-specific cadherin, γ-glutamyltranspeptidase, etc.) to provide spatially restricted gene inactivation or under the control of an inducible promoter (e.g., polyinosinic-polycytidylic acid/interferon induced Mx1, tamoxifen-induced estrogen receptor) to induce temporal gene inactivation, or both. Inducible *Pkd1* inactivation before murine postnatal Day 14 causes a very rapid and severe kidney cyst formation, whereas later inactivation causes delayed disease progression in adult mice {Torres 2007}.

Although the conditional models are viable and survive after birth for variable periods of time, they do not faithfully reflect human disease development. Affected kidney segments, dictated by the Cre promoter, may not correspond exactly to the distribution of the cysts in humans. The inactivation of the PKD1 or PKD2 gene in inducible knockouts occurs all at one time rather than sequentially which most likely occur in human ADPKD. Furthermore, the timing of inactivation and the dose of the inducing agent critically determine the severity of the phenotype and need to be tightly controlled to avoid excessive variability {Torres 2007}.

A number of knock-in mouse models have also been developed with hypomorphic (*Pkd1v, Pkd1nl, Pkd1l3, Pkd1RC*) or hypomorphic-like mutations (*Pkd2WS25*). The disease course in these animal models is slower, depends on the level of expression of functional polycystin-1 or polycystin-2, and is more suitable to test potential therapies for ADPKD {Torres 2007}.

Table 7 summarizes the models targeting or overexpressing PKD orthologs.

Table 7: Murine Models Targeting or Overexpressing PKD Orthologs {Torres 2007}

Strain	Mutation	Phenotype	Kidney Cysts	Pancreas cysts	Other	Pkd$^{+/-}$
Constitutive Pkd1 knockout mice						
Pkd1^{del1}	Exon 1 disruption	lethal	++	++	Edema	kidney/liver
Pkd1$^{del2-4LacZ}$	Exon 2-4 deletion with in-frame lacZ	lethal	++	++	----	----
Pkd1^{del4}	Exon 4 deletion	lethal	++	++	Edema, axial skeletal defects	kidney/liver/ pancreas
Pkd1^{del2-6}	Exon 2-6 Deletion	lethal	++	----	Edema, cardiac malformations	----
Pkd1$^{del17-21/geo}$	Exon 17-21	lethal	++	----	Edema, axial skeletal defects, cardiac malformations	kidney/liver
Pkd1^{del34}	Exon 34	lethal	+	+	Edema, axial skeletal	kidney/liver/

	deletion				defects	pancreas
$Pkd1^{del43-45}$	Exon 43-45 deletion	lethal	++	++	Edema, capillary leak	----
Conditional Pkd1 knockouts						
$Pkd1^{flox}$	KspCad-Cre	Rapid progression (death at 17 d)	+++	---	---	normal
$Pkd1^{flox}$	Pkhd1-Cre	Rapid progression (death at 35 d)	+++	---	---	normal
$Pkd1^{flox}$	γGT Cre-Cre	Rapid progression (death at 28 d)	+++	---	---	normal
$Pkd1^{flox}$	MMTV-Cre	Slow progression mild	+	----	----	normal
$Pkd1^{flox}$	Nestin-Cre	Intermediate progression	++	---	---	normal
Conditional (inducible) Pkd1 knockouts						
$i Pkd1^{flox}$	MX1-Cre	Variable depending on timing and dose of induction	+ - +++	---	Liver cysts	normal
$iKsp-Pkd1^{flox}$	KspCad-CreER	Variable depending on timing and dose of induction	+ - +++	---	normal	normal
Hypomorphic Pkd1 models						
$Pkd1^{V}$	T3041V knock-in (non-cleavable PC1)	Rapid progression, (death at14-42 d)	+++	No cysts		normal
$Pkd1^{nl}$	Exon 2-11 with aberrant splicing	Variable progression, 40% 1 mo 10% >1 yr	+++	+	Dissecting aneurysms	normal
$Pkd1^{L3}$	Aberrant transcription and/or splicing	Variable progression, 50% 1-2 mo 10% >1 yr	+++	+	---	Normal
$Pkd1^{RC}$	R3277C knock-in	Progressive cystic disease from E16.5 to 12	+++	No cysts	---	Normal

		months				
Transgenic models overexpressing Pkd1						
TPK1, TPK3	PKD1-TSC2 transgene	Variable	Glomerular cysts	---	---	---
Pkd1 TG	PKD1 transgene	Variable	+++	---	ICA, dilated aortic root, LVH	---
Constitutive Pkd2 knockouts						
Pkd2-	Exon disruption	Lethal	+	+	Edema, laterality defects	Kidney/liver
Pkd2-LacZ	Exon 1 deletion LacZ "promoter trap"	Lethal	+	+	Edema, laterality defects	---
Conditional Pkd2 knockout						
Pkd2$^{flox2-4}$	pCxCreER^{TM}	Slow progression	No cysts	---	---	Liver
Hypomorphic Pkd2 models						
Pkd2^{WS25}	Exon 1 duplication with disruption	Slow progression	+	+	---	Kidney/liver
Transgenic models overexpressing Pkd2						
Pkd2-Y	PKD2-ABCG2 transgene	Slow progression	Micro-cysts		Mild nephrogenic diabetes insipidus	---
PKD2 TG	PKD2 transgene	Variable	++	---	---	---

Several rodent PKD models have been used to test potential therapies {Torres 2007}. The ideal animal model should be genetically orthologous and reproduce the typical phenotype of human ADPKD. However, few, if any, of these animal models, meet all these requirements. For this reason it seems prudent to confirm the potential benefits of an experimental drug in more than one animal model. Such an approach has been used successfully in the preclinical development of tolvaptan, as discussed below. **Table 8** contains a non-exhaustive list of therapies and models tested in preclinical trials {Torres 2007}.

Table 8: Effectiveness of selected therapeutic interventions in animal models of Polycystic Kidney Disease {Torres 2007}

	Rats		Mice									
	Han: SPRD	**PCK**	**orpk**	**bpk**	**cpk**	**Jck**	**pcy**	*Pkd1$^{-/-}$*	*Pkd1$^{c/c}$*	*Pkd1^{hyp}*	*Pkd2$^{c/c}$*	*Pkd2$^{-/WS25}$*
Protein restriction	yes	----	----	----	----	----	yes	----	----	----	----	----
Soy-based protein	yes	----	----	----	----	----	yes	----	----	----	----	----
Flax seed	yes	----	----	----	----	----	----	----	----	----	----	----

Bicarbonate/citrate	yes	no	----	----	----	----	no	----	----	----	----	no
Paclitaxel	no	----	no	----	yes	----	no	----	----	----	----	----
Methylprednisolone	yes	----	----	----	----	----	yes	----	----	----	----	----
Triptolide	----	----	----	----	----	----	----	yes	yes	----	----	----
TRPV4 activator	----	yes	----	----	----	----	----	----	----	----	----	----
Calcimimetics	yes	no	----	----	----	----	yes	----	----	----	----	no
V2R antagonist	----	yes	----	----	yes	----	yes	----	yes	----	----	yes
SST analogs	---	yes	----	----	----	----	----	----	----	----	----	yes
c-Src inhibitor	----	yes	----	yes	----	----	----	----	----	----		----
Raf inhibitor	no	----	----		----	----	----	----	----	----	no	----
MEK inhibitor	----	----	----	----	----	----	yes	----	no	----	----	----
Rapalogues	yes	----	yes	yes	----	----	yes	----	yes	yes	yes	yes
Metformin	----	----	----	----	----	----	----	----	yes	----	----	----
PRARγ agonist	yes	yes	----	----	----	----	----	yes	no	----	----	----
HDAC inhibitors	----	----	----	----	----	----	----	yes	yes	----	----	----
CDK inhibitors	----	----	----	----	yes	----	----	----	----	----	----	----
Cdc25A inhibition	----	yes	----	----	----	----	----	----	----	----	----	yes
c-myc antisense	----	----	----	----	yes	----	----	----	----	----	----	----
TNFα inhibition	----	----	----	yes	----	----	----	----	----	----	----	yes
STAT3 inhibitors	----	----	----	----	----	----	----	----	yes	----	----	----
STAT6 inhibitors	----	----	----	yes	----	----	----	----	----	----	----	----
EGFR TK inhibitor	yes	no	yes	yes	----	----	----	----	----	----	----	----
ErbB2 TK inhibitor	----	yes	----	yes	----	----	----	----	----	----	----	----
VEGFR inhibitor	yes	----	----	----	----	----	----	----	no	----	yes	no
GlucCer synth inh	----	----	----	----	----	yes	yes	----	yes	----	----	----

Abundant evidence for renal functional deterioration in association with enlargement of TKV has been shown in multiple animal models {Grantham 2006b, Torres 2008}. The rates of renal enlargement and

renal function decline are faster in rodent models of PKD than in humans. As in human ADPKD, kidney enlargement in these animal models consistently precedes the development of renal insufficiency.

Table 9 summarizes the results of studies in which measurements of renal volume and function were made in control animals and animals that were treated with several different regimens. Treatments were usually started just after the animals were weaned and maintained for several weeks. Improvements in renal volume and function were evaluated by comparing the kidney weights (KW) and functional parameters of treated and untreated cystic animals to wild-type counterparts that served as age- and sex-matched controls. Treatments that inhibited renal enlargement consistently reduced the rate of renal function decline. The changes in kidney volume resulting from the different treatments correlated reasonably well with the changes in renal function {Grantham 2006}.

Table 9: Relative beneficial effect of various interventions on kidney volume

Study	% Improved KW	% Improved BUN	Model	Duration
Soy vs. casein protein	27.4	70[a]	Han:SPRD, M	3 to 10 w
Enalapril, 50 mg/L po[c]	22.8	43.9[a]	Han:SPRD, M	3 to 16 w
Enalapril, 50 mg/L po	31.0	74.2[a]	Han:SPRD, M	3 to 10 w
Enalapril, 50 mg/L po	32.7	48.1[b]	Han:SPRD, M	3 to 40 w
Losartan, 400 mg/L po	12.3	63.4[a]	Han:SPRD, M	3 to 16 w
Lovastatin, 4 mg/Kg per day ip	21.7	58.8	Han:SPRD, M	4 to 10 w
Methylprednisolone, 1-2 mg/Kg per d po	65.7	74.0	pcy	4 to 18 w
Methylprednisolone, 1-2 mg/Kg per d po	33.1	40.1	Han:SPRD, M	3 to 10 w
WTACE2, 100 mg/kg per d ip	46.7	54.8	bpk	7 to 21 d
EKI-785, 90 mg/Kg q3d ip	66.7	100.0	bpk	7 to 24 d
EKI-785, 90 mg/Kg q3d ip	85.5	100.0	bpk	7 to 21 d
EKI-785, 90 mg/Kg q3d ip	21.2	41.8	Han:SPRD, M	3 to 10 w
EKB-569, 90 mg/Kg q3d ip	75.2	94.8	bpk	7 to 21 d
EKB-569, 30 mg/Kg q3d ip + WTACE2 100 MG/Kg altd ip	74.3	94.8	bpk	7 to 21 d
EKB-569, 20 mg/Kg q3d ip	38.1	59.5	Han:SPRD, M	3 to 10 w
c-myc antisense oligomer, 30 mcg/d ip	36.7	66.0	cpk	21 d
Rapamycin, 0.2 mg/Kg per d ip	64.6	84.6	Han:SPRD, M	3 to 8 w
OPC-31260, 100-200 mcg per d sq	54.4	86.4	cpk	3 to 21 d
OPC-31260, 0.1% po	86.2	62.2	pcy	4 to 30 w

| OPC-31260, 0.1% po | 75.0 | 95.9 | PCK | 3 TO 10 w |
| OPC-31260, 0.05% po | 98.4 | 99.5 | Pkd2-/WS25 | 3 TO 16 W |

[a]Data are based on sCr values
[b]Data are based on insulin clearance
[c]Per Os – meaning Oral Administration

Animal models have been employed in the development of the vasopressin V2 antagonist tolvaptan. The rationale for targeting the vasopressin V2 receptor to treat PKD includes: 1) Cyclic AMP plays a central role in the pathogenesis of PKD through disruption of tubulogenesis and stimulation of cell proliferation and chloride driven fluid secretion, 2) Vasopressin acting on V2 receptors is the most powerful agonist for cAMP generation in freshly isolated collecting ducts, 3) Nearly exclusive localization of V2R on collecting ducts, connecting tubules, and thick ascending limbs of Henle, the main sites of cystogenesis, minimizes off target toxicities, 4) Vasopressin is continuously present in the circulation, likely at a higher level in PKD to compensate for a urinary concentrating defect, and 5) Cyst development is almost completely inhibited in PCK rats lacking circulating vasopressin (generated by crosses of PCK and Brattleboro rats), while administration of the V2R agonist 1-deamino-8-d-arginine vasopressin (dDAVP) fully rescues the cystic phenotype. In 1999, Gattone *et al.* {Gattone 1999} reported that the V2 receptor antagonist OPC-31260 had a marked protective effect on the development of PKD in the cpk mouse, a model of rapidly progressive cystic disease. To extend this observation, OPC-31260 was then used in three animal models orthologous to human ARPKD (PCK rat), ADPKD (*Pkd2WS25/_* mouse), and adolescent nephronophthisis (*pcy* mouse) {Gattone 2003, Torres 2004}. In PCK rats, the administration of OPC-31260 between 3 and 10 weeks or between 10 and 18 weeks of age significantly reduced the renal levels of cAMP, the activation of Ras and extracellular signal–regulated kinase, and the expression of the pro-proliferative isoform of B-Raf. This was accompanied by a marked inhibition of disease development, when administered between 3 and 10 weeks of age, or of disease progression, when administered between 10 and 18 weeks of age, as reflected by significant reductions in total kidney volume, cyst and fibrosis volumes, plasma blood urea nitrogen (BUN), and mitotic and apoptotic indices. In *Pkd2WS25/_* mice, the administration of OPC-31260 lowered the renal levels of cAMP, downregulated the expression of V2 receptor– and cAMP-dependent genes (V2 receptor and aquaporin 2) and markedly inhibited the development of PKD, as reflected by lower kidney volume, cyst and fibrosis volumes, plasma BUN levels, and mitotic and apoptotic indices. OPC-31260 has been also effective in a conditional *Pkd1* knockout when treatment is started early following gene deletion. Because OPC-31260 is a weak antagonist for the human V2 receptor, a derivative with a higher affinity for the human V2 receptor (tolvaptan) was evaluated. This antagonist was also effective in animal models of ARPKD, ADPKD, and nephronophthisis {Wang 2005, Gattone 2005, Wang 2005b). Neither OPC-31260 nor tolvaptan had a beneficial effect on the development of fibropolycystic liver disease, which is consistent with the absence of V2 receptor expression in the liver.

Thus, evidence has been provided both in terms of natural history and in therapeutic interventions using a vasopressin V2 receptor antagonist that expansion of TKV in preclinical models is highly associated with renal functional deterioration and fibrosis that is reversible with blockade of the V2 receptor antagonist.

3.6   Total Kidney Volume: Summary of Clinical Trials and TKV Outcome

There is an increasing and impressive body of evidence demonstrating that the kidneys of patients with ADPKD progressively increase in size from birth to the sixth decade of life, and the clinical symptoms and signs of ADPKD including hypertension, gross hematuria, flank and abdominal pain, and declining GFR are associated with TKV and the rate of kidney growth.

The earliest determinations of TKV in patients with ADPKD were performed by CT in 1981 {Thomsen 1997}. TKV was calculated by summing the surface area of contiguous 13 mm CT slices in 43 patients with ADPKD. A moderate inverse correlation (R = –0.473) between combined kidney volume and creatinine clearance was observed.

The first longitudinal study of changes in TKV in patients with ADPKD was published in 1992 {Gabow 1992}. In this study, 42 serial ultrasonographic measurements of TKV spanning 8.3 years from birth were made in a child with ADPKD. Bilateral kidney volume increased steadily and asymmetrically throughout the study period. The asymmetry was ascribed to the differential burdens of renal cysts at birth.

In 2000, two studies analyzed serial TKV measurements from CT images. The first was a prospective study following nine ADPKD patients over a mean period of eight years {King 2000}. TKV increased at a mean rate of 4.0% per year. The second was a retrospective study of ten ADPKD patients followed for a mean of 5.7 years {Sise 2000}. TKV increased at a mean rate of 9.4% per year. The rates of increase in TKV were highly variable. Patients with fast rates of growth exhibited more serious declines in GFR than patients who exhibited slower growth rates.

In 2001, sequential measurements of TKV determined by ultrasonography between birth and 20 years of age in 182 children with ADPKD were reported {Fick-Brosnahan 2001}. The rate of increase in kidney volume in children with ADPKD was ~10.3% per year, although, as inferred from the organ growth observed in individuals without ADPKD, a sizeable portion of that increase was due to physiological parenchyma growth (~9.5% per year). The differences in absolute kidney size observed as the patients with ADPKD aged can be attributed to the volume of the cysts. Although this difference in TKV was relatively small at birth, it was magnified by the sustained exponential growth of the cysts. This study also showed that the absolute size and the rate of kidney growth were significantly correlated with the elevation of blood pressure above the 75th percentile.

In 2002, the same research team reported the first large-scale, sequential, quantitative ultrasonographic measurements of TKV in 229 adults with ADPKD over a mean interval of 7.8 years {Fick-Brosnahan 2002}. The mean rate of TKV increase was 8.2% per year. This rate was slightly lower than that observed in children. Inverse correlations between TKV and GFR and between the rate of increase in TKV and the rate of change in GFR were observed. These associations suggest a potential link between the growth of cysts and decline in renal function.

The CRISP study, consisting of an initial three-year study (CRISP I) and a five-year follow-up study (CRISP II), is the largest systematic, longitudinal study of TKV and renal cyst volume progression in patients with ADPKD utilizing MRI. In this study, 241 adults with ADPKD who had creatinine clearance >70 ml/min/1.73 m^2 underwent annual MRI measurements of TKV and total cyst volume. TKV was

measured using the stereology method from gadolinium-enhanced T1-weighted MRIs in CRISP I, and total cyst volume was measured using a region-growing segmentation method from T2-weighted MRIs {Grantham 2006a}. The mean combined volume of both kidneys of participants at baseline was 1,060 ml and the mean combined total cyst volume (TCV) was 540 ml; TKV increased at a rate of 5.3% per year and total cyst volume by 12.0% per year with total cyst volume measurements being much more variable. The relationship between age and TKV or TCV over the three-year interval was exponential, implying that the volume enlargement process is driven by tissue growth. In addition, the correlation between TKV and TCV was r = 0.95 {Grantham 2006a}. The left and right kidneys enlarged at similar rates and, in each patient, the cysts seemed to grow at relatively constant rates over the first four decades of life. Subsequent analysis revealed that the number of cysts formed in each kidney was an important determinant of TKV and TCV {Harris 2006}, although the growth rate of individual cysts seemed to be the most powerful determinant of how fast the kidneys would enlarge {Grantham 2010}. An inverse correlation (R = –0.37) was observed between TKV and GFR at baseline. Notably, GFR declined significantly (by 4.33 ± 8.07 ml/min/1.73m^2) during the first three years of follow-up in those subjects whose TKV exceeded 1500 ml, more than five-fold normal. Subjects with TKV<1500 ml had non-significant changes in GFR during the three-year period of study.

The reliability and variability of repeated TKV measures were determined by repeated measurements performed on MRIs of phantoms and four repeat measures on four human participants. In addition, consequent to the labeled "Boxed Warning" on the use of gadolinium contrast agents in patients with reduced GFR due to concerns of nephrogenic systemic fibrosis, the reliability and accuracy of MRI-based measurements of TKV without gadolinium was established {Bae 2009}. The effects of gadolinium on TKV measures have been published, and show a slightly greater TKV measure in those receiving gadolinium that impacted smaller kidney volume measurements the most (4 vs. 2.1% in those with larger kidneys) {Bae 2009}.

The CRISP II study extended the preliminary observations of CRISP I over an additional five-year period. This follow-up study aimed to determine if the initial baseline height-adjusted TKV (htTKV) predicted the onset of renal insufficiency defined as CKD Stage. In CRISP II, gadolinium-enhanced MRI was not used, based on emerging concerns of a potential association between nephrogenic systemic fibrosis (NSF) in patients with compromised renal function. 201 CRISP I participants were re-enrolled in CRISP II. After 7.9+/- 0.6 years of follow up, Stage 3 CKD occurred in 30.7% of the enrollees. Using baseline htTKV, negative correlations with GFR increased from -0. 22 at baseline to -0.65 at year 8 in those who had complete follow-up. In multivariate analysis, a baseline htTKV increase of 100 cc/m significantly predicted the development of Stage 3 CKD within eight years with an odds ratio of 1.48 (95% confidence interval: 1.29, 1.70). In receiver operator characteristic curve analysis, baseline htTKV of 600cc/m most accurately defined the risk of developing Stage 3 CKD within eight years with an area under the ROC curve value of 0.84 (95% confidence interval: 0.79, 0.90). HtTKV was determined to be a better predictor than baseline age, sCr, BUN, urinary MCP1 or urinary albumin excretions. The authors concluded that these results support the use of TKV as a prognostic biomarker {Chapman 2012}.

Following the initial CRISP observations, investigators in Korea used MRI to determine kidney volume and kidney cyst volumes in 56 patients with ADPKD and reported that TKV and TCV were inversely correlated with creatinine clearance {Lee 2006}.

A summary of the relationship between kidney volume and clinical variables is in **Table 10** below
{Grantham 2006b}.

Table 10: Relation between kidney volume and clinical variables

Variable	Number studied	Volume method	Mean Kidney Volume[a]			
			With variable	Without variable	P Value	Reference
Proteinuria	270	US	1190±93	578±32	<0.0001	Grantham 1996
Microalbuminuria	49	US	853±87	535±52	<0.01	Grantham 1996
Hypertension						
Males	76	US	624±47	309±43	<0.0005	Gabow 1992
Females	89	US	446±32	338±24	<0.002	Gabow 1992
Hypertension	43	CT	976±472	739±311	<0.05	Chapman 1991
	241	MR	628±48	352±33	<0.0001	Fick-Brosnahan 2002
Hypertension children	70	US	125±7	83±6	<0.0001	Segura 1996 Fick 1994
Gross hematuria	191	US	820±87	588±52	<0.03	Gabow 1993
Progressive loss of renal function	43	CT	895[c]	606[c]		Chapman 1991
	220	US	598±368	366±168	<0.0001	Grantham 1997

[a]Mean kidney volume is combined kidney volume ÷2 (ml)
[b]Kidney volume corrected for body size
[c]Derived from combined kidney volume data

In a study of 71 normotensive and 76 hypertensive ADPKD patients with creatinine clearances greater
than 75 ml/min per 1.73 m^2, hypertensive patients were found to have significantly greater kidney volume
compared with normotensive counterparts {Gabow 1990a}. A similar relationship was found between
hypertension and cystic involvement in children with ADPKD {Fick 1994}. These studies support the
proposal for renal structural involvement as a factor in hypertension in ADPKD {Ecder 2001b}.
Improvement in blood pressure in response to percutaneous cyst aspiration as a result of refractory pain
also supports the role of cystic compression in the development of hypertension in these patients {Bennett
1987}. A recent study demonstrated that hypertensive children with ADPKD had a greater increase in
fractional cyst volume over time and more rapidly increasing cyst number compared with normotensive
children with ADPKD {Cadnapaphornchai 2011}.

The first interventional treatment study of adults with ADPKD reported the use of serial CT
measurements to determine if administration of octreotide for six months would reduce the rate of
increase in TKV {Ruggenenti 2005}. All 12 participants of this pilot study had TKV in excess of 1,200

ml and GFRs below 60ml/min/1.73 m^2. At baseline, mean TKV, measured by semi-automated segmentation and summation of contiguous slices, was 2,435 ml for all participants and increased by an average value of 162 ml over the next six months (11.8% volume increase per year) in the control group and 71 ml (4.4% volume increase per year) in the octreotide-treated group. TCV at baseline, determined with a semi-automated method on the basis of CT attenuation histogram analysis, was 1,631 ml and increased by an average value of 212 ml in controls versus 61 ml in the octreotide-treated group.

A five-year controlled trial to determine the effect of ACEI on ADPKD progression in children was published in 2009 {Cadnapaphornchai 2009}. Serial measurements of TKV were conducted using the ellipsoid formula to analyze images obtained by ultrasonography. TKV increased exponentially as would be expected in growing children with an increase of 9.3% per year across all groups. The relationship between TKV and hypertension was striking in this study, and the rate of kidney growth was increased in hypertensive patients. The likely explanation is that children with hypertension who had larger kidneys harbored more cysts in their kidneys than those with normal blood pressure. Treatment with ACEI had no measurable effect on TKV but an improvement in GFR and in left ventricular mass index was evident.

Several studies that use quantitative imaging to shorten the time required to evaluate the efficacy of new treatments for ADPKD have been completed. A six-month period of observation was also examined in an MRI study of 100 adults with ADPKD in Switzerland {Kistler 2009a}. In this study, TKV was measured using manual delineation of the kidney contour on contiguous slices of T1-weighted, gadolinium-enhanced MRIs. Values for TKV and TCV were similar to those recorded in the CRISP study, as were the annual rates of TKV and TCV change. TKV at baseline was positively associated with baseline hypertension, gross hematuria, and GFR. The researchers of this study observed that the asymptomatic rupture of large cysts in seven patients was associated with significant reductions in TKV and recommended that detailed image analysis be performed in any study of ADPKD progression over a relatively short interval.

The mammalian target of rapamycin (mTOR) is a serine protein kinase that coordinates cell growth, cell-cycle progression and proliferation. Aberrant mTOR activation and signaling is thought to play a role in the pathogenesis of ADPKD {Fingar 2004, Shillingford 2006}. The mTOR inhibitors sirolimus and everolimus have been shown to retard cyst growth and preserve renal function in rodent models {Soliman 2009, Wu 2007}. Administration of sirolimus to ADPKD patients after renal transplantation was associated with a reduction in native kidney cystic volume {Shillingford 2006}.

A pilot study was performed on eight adult patients with ADPKD who received sirolimus and telmisartan, an angiotensin receptor blocker, for six months. An additional eight ADPKD control patients received only telmisartan. There was no change in kidney volume as measured by MRI in the sirolimus group compared with a significant increase in the control group. Renal function was stable in five out of eight subjects in the sirolimus group, improved in two patients, and worsened in one. In the control group, renal function was stable in three patients, improved in two cases, and worsened in three. The authors concluded that although prospective longer term studies were needed, sirolimus was promising as a potential treatment for ADPKD {Soliman 2009}.

The SIRENA study compared six months' treatment with sirolimus or conventional therapy on the growth of TKV in 21 patients with ADPKD and GFR ≥40 ml/min/1.73m^2. Fifteen patients completed the study. TKV increased less on sirolimus than on conventional therapy, but this was not statistically

significant. TCV was stable on sirolimus and increased in the control group, whereas parenchymal volume increased with sirolimus and was stable in the control group. Sirolimus had no appreciable effect on GFR. However, albuminuria and proteinuria increased significantly in this group. The authors concluded that six months of sirolimus therapy halted the growth of renal cysts and increased the volume of seemingly healthy kidney parenchyma in this small proof-of-concept study {Perico 2010}.

The SUISSE ADPKD study was a larger, 18-month, single center, open-label, randomized, controlled trial in 100 ADPKD patients between 18 and 40 years of age. Median TKV at randomization was 907 cm^3. After 18 months of treatment, low dose sirolimus did not halt TKV progression (P=0.07). GFR did not differ significantly between the two groups; however, urinary albumin excretion rate was higher in the sirolimus group, consistent with other reports of this class of drug {Serra 2010, Chapman RJ 2010}. Given the positive results with sirolimus in mouse models, the authors postulated that therapy was likely administered at much earlier stages of cyst development in the rodent studies, and that these models may not appropriately reflect the more complex and heterogeneous pathogenesis of ADPKD in humans. In addition, the dose of sirolimus may have been suboptimal. The achieved dose was about 25% lower than the intended dose mainly because of side effects {Serra 2010}. This meant that the doses used in this study were less than needed to reach the circulating levels found in preclinical models {Torres 2010}.

The effect of everolimus was studied in a two-year, multi-center, randomized, double-blind, placebo-controlled trial in 433 ADPKD patients with stage II or III chronic kidney disease (eGFR 30-89ml/min/1.73m^2) {Walz 2010}. The primary outcome was change in TKV as measured on MRI at 12 and 24 months. Baseline TKV was 2028±1173 ml in the everolimus group and 1911±1153 ml in the placebo group. Compared to placebo, everolimus slowed the increase in TKV at year 1 (P<0.04) with a trend in slowing at year 2 (p=0.06), but did not slow the progression of renal impairment. In the everolimus group, eGFR improved during the first three months before declining in subsequent months. The authors postulated that increased mTOR activity may not be detected in all cysts, and that rapid shrinkage of susceptible cysts during the first months of treatment may explain the short-term increase seen initially in renal function. Unfortunately, MRI assessment of TKV was not performed at this very early time point. In addition to evident proteinuric effects of the drug, the everolimus group experienced an increased frequency of side effects, including an increased rate of peripheral edema, and consequent use of diuretics may also have negatively affected renal function. There was also a drop-out rate of approximately 33% in the everolimus group with analyses performed in an intent-to-treat fashion. They acknowledged that this trial was conducted in patients with advanced cystic disease and fibrosis that could potentially be irreversible and thus unresponsive to therapies that could improve renal function, obscuring potential benefits in ADPKD patients with earlier disease. With regard to the suitability of TKV for assessing outcomes of certain therapeutic interventions or in ADPKD patients with large kidneys and renal dysfunction {Walz 2010}, the significant hemodynamic and proteinuric specific side effects independent of ADPKD seen with everolimus in the setting of potential benefits with regard to cyst growth make this difficult to interpret.

Grantham, Bennett, and Perrone responded to the publication of these two trials highlighting the inclusion of patients with late-stage ADPKD in the everolimus trial, and the suboptimal dose of sirolimus in the SUISSE study. They concluded that the use of TKV and TCV as surrogate markers of disease progression informed both of these trials and that the use of these measures in clinical trials involving

patients with early stage ADPKD is rational and supported by strong scientific evidence {Grantham 2011a}.

Higashihara and Torres recently published combined results from a Japanese and North American three-year open-label trial with tolvaptan, a V2-specific vasopressin receptor antagonist, in 63 ADPKD patients who were randomly matched 1:2 to historical controls. TKV increased by 5.8% in controls compared with 1.7% in the tolvaptan treated patients. Annualized eGFR declined by 2.1 ml/min/1.73m^2 in the control group and by 0.71 ml/min/1.73m^2 in the tolvaptan-treated patients. Increasing TKV correlated with decreasing eGFR {Higashihara 2011}.

The TEMPO ¾ (NCT00428948) has been completed and is discussed below. Bosutinib (NCT01233869) and HALT PKD (NCT00283686) clinical trials are on-going. These three studies have incorporated TKV as a primary endpoint {clinicaltrials.gov}.

The effect of tolvaptan was studied in a three-year, multi-center, randomized, double-blind, placebo-controlled trial in 1445 ADPKD patients with an estimated creatinine clearance of 60 ml/min/1.73m^2 or more and TKV of 750 ml or more {Torres NEJM 2012a}. Subjects 18 to 50 years of age were randomized in a 2:1 ratio to receive tolvaptan, a V2-receptor antagonist, at the highest of three twice-daily dose regimens that the patient found tolerable, or placebo. The primary outcome was the annual rate of change in the TKV. Sequential secondary end points included a composite of time to clinical progression (defined as worsening kidney function, kidney pain, hypertension, and albuminuria) and rate of kidney-function decline. Over a three-year period, the increase in TKV in the tolvaptan group was 2.8% per year (95% confidence interval [CI], 2.5 to 3.1), versus 5.5% per year in the placebo group (95% CI, 5.1 to 6.0; P<0.001). The composite end point favored tolvaptan over placebo (44 vs. 50 events per 100 follow-up-years, P = 0.01), with lower rates of worsening kidney function (2 vs. 5 events per 100 person-years of follow-up, P<0.001) and kidney pain (five vs. seven events per 100 person-years of follow-up, P = 0.007). Tolvaptan was associated with a slower decline in kidney function (reciprocal of the serum creatinine level, −2.61 [mg per milliliter]$^{-1}$ per year vs. −3.81 [mg per milliliter]$^{-1}$ per year; P<0.001). There were fewer ADPKD-related adverse events in the tolvaptan group but more events related to aquaresis (excretion of electrolyte-free water) and hepatic adverse events, contributing to a higher discontinuation rate (23%, vs. 14% in the placebo group).

Tolvaptan, as compared with placebo, slowed the increase in TKV and the decline in kidney function over a three-year period in patients with ADPKD. Preservation of GFR was associated with a smaller increase in TKV.

Thus, TKV remains a rational marker for disease progression and assessment of interventional therapy in patients with early stage ADPKD. However, when used to assess the effect of interventional therapy, TKV will need to be assessed in the context of the investigational agent's mechanism of action and its potential for nephrotoxicity or other side effects that could impact renal function.

3.7 Systematic Literature Review of ADPKD Natural History

A literature search was conducted to determine what previous studies had published relating to the natural progression of ADPKD and what was known about TKV and its relationship to the clinical outcome events.

The literature review was performed in three levels as explained in **Figure 6**. First, six MEDLINE searches were carried out in September, 2012 (see **Table 11**) to capture all abstracts and articles relevant to the topic of Epidemiology/Natural history of ADPKD and its outcomes including the relationship of TKV to outcomes. This search encompassed all types of articles, but editorials, letters, abstracts, unpublished reports, reviews, and articles published in non-peer-reviewed journals were not used in the analysis. The Steering Committee also decided to exclude publications from journal supplements because of potential differences in the process of how they get solicited, selected, reviewed, and edited compared with peer-reviewed publications in main journals. The MEDLINE searches for relevant terms included polycystic kidney disease, autosomal dominant polycystic kidney disease, natural history, kidney volume, total kidney volume, and epidemiology. The searches were limited to English language publications since 1990. There were no meta-analyses or relevant systematic reviews to draw upon. The first three searches were relatively broad and encompassed all cystic kidney diseases resulting in too many documents to reasonably review and were further refined in subsequent searches to focus on ADPKD. Searches numbered four, five, and six resulted in 252 articles for consideration. Search #6 produced 24 articles that were all duplicates of those in search #5, resulting in 228 unique articles for further review.

The second level of review was done by looking at the abstracts of these 228 articles, removing those that did not meet the inclusion criteria review, resulting in 143 articles for detailed analysis. These articles were tabulated by citation, population, number of individuals, follow-up time, study design (cross-sectional or longitudinal, prospective or retrospective), and by predictors and outcomes of interest. These articles were tabulated into overview tables (Appendix 8.3) by the reviewers and interpreted by the Steering Committee members. In level three, all 143 articles were read, and 90 of those met the criteria and were used in the analysis. The results of the analysis are given below.

Natural History of ADPKD

The specific questions to be addressed by the systematic review were:

1. What is the natural history of ADPKD? What is the impact of different demographics, including age, genotype, gender, and ethnicity on natural history of ADPKD?

2. What clinical outcomes are associated with ADPKD? How are these evaluated?

3. What is the relationship of standard markers of renal function and clinical outcomes, including cardiovascular complications and death?

The systematic review of articles addressing the natural history of ADPKD yielded articles which were of low to moderate quality, small, primarily descriptive and cross-sectional, and neither longitudinal nor prospective. All of this literature is well known to members of the Steering Committee and clinicians who care for patients with ADPKD; these results represent our current understanding of the complications of ADPKD, including the development of CKD and ESRD, the development of complications including hypertension, hematuria, pain, liver cysts, intracranial aneurysms, and seminal vesicle cysts, and the effect of genotype and gender. These concepts are already well summarized in sections 3.4.3 Natural History and 3.4.5 Current Standard of Care. The relationship of standard markers of renal function and clinical outcomes is not precisely delineated in any of the reviewed studies, but in general, complications and clinical outcomes are associated with worse renal function. Due to the descriptive and cross sectional nature of the literature, precise linkage between age, GFR, and complications could not be made.

The specific questions on the relationship of TKV in ADPKD to be addressed by the systematic review were:

1. How was TKV evaluated? What is the sensitivity and specificity of imaging tests for TKV (US, CT, MRI)?

2. How is TKV associated with the natural history of ADPKD?

3. What is the relationship of TKV and clinical outcomes, including cardiovascular complications and death?

The search results pertaining to evaluation of TKV including sensitivity and specificity of imaging tests for TKV yielded relatively recent publications which had previously been summarized in section 3.6 Total Kidney Volume: Summary of Clinical Trials and TKV Outcome. The search results pertaining to association of TKV and natural history including clinical outcomes yielded relatively few publications. Almost all of these were cross-sectional and descriptive. Perrone *et al.* (HALT CV paper) showed an association of TKV with left ventricular mass, although left ventricular hypertrophy was present in only a few of these subjects with relatively young age (mean ~36) and preserved kidney function (eGFR >60 ml/min/1.73 m^2 at study entry). Torres *et al.* demonstrated that larger TKV was associated with a lower renal blood flow which proceeded the fall in GFR (Torres, KI 2012b, HALT baseline data). The longitudinal follow-up of the CRISP cohort demonstrated that baseline TKV of individuals with preserved kidney function was highly predictive of future kidney function decline (Chapman 2012). This paper is fully summarized in section 3.6 Total Kidney Volume: Summary of Clinical Trials and TKV Outcome. There were no publications that studied a relationship between TKV and ESRD or cardiovascular complications or death. The CRISP2 study (Chapman, 2012), had very few deaths or ESRD events, and thus did not have the power to examine a relationship between TKV and these events.

The paucity of large, well-powered clinical trials or prospective cohorts that address the relationships between TKV and clinical complications including ESRD or death represents a major deficiency in the current understanding of ADPKD. The analyses performed in this briefing book provide substantial insight in this regard, and demonstrate a substantial predictive power of TKV to predict clinical events including ESRD, and lesser degrees of renal function decline.

Table 11: MEDLINE Search Results

Search #	Topic	Number of Articles in PubMed
1	"Kidney Diseases, Cystic"	4401
2	"Polycystic kidney disease"	4806
	Limit to (English language)	4414
	Limit to (English language and humans)	3460
3	"Polycystic Kidney Disease" and ("Epidemiology" or "natural history")	390
	Limit to (English language)	351
	Limit to (English language and humans)	334
4	"Autosomal Dominant Polycystic Kidney Disease"	2179
	Limit to (English language)	2021
	Limit to (English language and humans)	1733
	"Autosomal Dominant Polycystic Kidney Disease" and ("Epidemiology" or "natural history")	210
	Limit to (English language)	190
	Limit to (English language and humans)	**185**
5	"Autosomal Dominant Polycystic Kidney Disease"	2179
	Limit to (English language)	2021
	Limit to (English language and humans)	1733
	"Autosomal Dominant Polycystic Kidney Disease" and ("kidney volume")	50
	Limit to (English language)	50
	Limit to (English language and humans)	**43**
6	"Autosomal Dominant Polycystic Kidney Disease" and ("Total kidney volume")	30
	Limit to (English language)	30
	Limit to (English language and humans)	**24**

Figure 6: Decision Tree for Systematic Literature Review

2,179
Publications identified from literature search "Autosomal Dominant Kidney Disease"

252
Level 1 Review:
Publications remaining after including electronic search parameters:
- "Epidemiology" or "Natural History" + "Kidney Volume" + "Total Kidney Volume"
- English language
- Human

*Table 17, Searches 4-6. Searches 1-3 were discarded as explained in text above.

228
Abstracts reviewed.
*24 articles were discarded due to duplication between Search 5 and 6

143
Level 2 Review:
Abstracts remaining after screening for Exclusion/Inclusion Criteria:
- Publications prior to 1990
- Animal Model and In-Vitro Studies
- Case reports, letters, comments, reviews, editorials, meta-analyses
- Does not include TKV information

143
Full text publications retrieved for detailed evaluation

53
Level 3 Review:
Full text publications excluded:
- ESRD/Dialysis Study (7)
- No Natural History or Information on Total Kidney Volume (12)
- Genetic Analyses (9)
- Extrarenal focus, No information on Total Kidney Volume (11)
- Study Design or Method Description only (2)
- Did not meet Level 1 or 2 criteria (Letter to the Editor) prior to 1990, etc) (13)

90
Publications accepted for data extraction. See Appendix 8.3

A copy of the Data Extraction Table is provided in Appendix 8.3 and the full list of 'Excluded' and 'Included' publications (from the search process outlined above) is provided in Appendix 8.4.

4   Methods – Qualification Research Plan for TKV

4.1   Data Sources

Given the paucity of available clinical data from ADPKD patients and the limited number of clinical trials involving small numbers of patients, the PKDOC and the FDA agreed to aggregate observational data from both patient registries and CRISP cohort studies. This enabled analyses to be performed on an expanded dataset which strengthen the ability to support the proposed context of use statement for TKV. Thus, de-identified observational data from the following five sources has been aggregated into a common database in a standard CDISC SDTM structure:

- University of Colorado – Denver

- Mayo Clinic

- Emory University

- Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease 1 (CRISP1)

- Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease 2 (CRISP2)

The content of these databases are described in detail below. With funding from the PKD Foundation and direction from experts at C-Path, each research organization has mapped their data into this structure with the aid of available translation tools. Once translated, the data were electronically uploaded via a secure connection to the C-Path online data repository. Before being added to the database, submitted data were validated using the OpenCDISC validator, an open source tool that ensures data sets conform to CDISC standards (www.opencdisc.org).

The data are stored at DataPipe, Inc., an industry-leading national data hosting company (http://www.datapipe.com). They provide the system hardware and internet connections. The hardware has redundant storage to maintain availability and data are backed up daily to prevent data loss in the event of a hardware failure. Availability, security, and performance are continuously monitored. The database is protected with secure, industry standard firewalls, protocol encryption, anti-service attack mechanisms, and forward and backward proxies. Read access to the data is handled via an application security layer that provides single sign-on logins, secure remote access, strong authentication mechanisms, and audit trails of user actions and data changes.

The common database contains data from a total of 2355 patients with ages ranging from 0-84 years at study entry (mean 36.1; median 37.7). Almost two-thirds (59.3%) of patients are female, and the majority (82%) are Caucasian. There were 242 deaths and 668 ESRD events with timing information. Additional descriptive statistics can be found in Figure 7 through Figure 22.

4.1.1 University of Colorado Database

Between 1985 and 2004, 5,684 individuals from 1228 families with ADPKD were recruited for ongoing natural history and genetic studies of ADPKD at the University of Colorado. These studies were continuously funded by NIH until 2008 (PO1 DK34039). IRB approval has been continuous since 1985. One or more affected members of each family were examined during a two-day evaluation at the University of Colorado General Clinical Research Center, at which time medical and family history was obtained and complete physical examinations were performed, including BP measurements. Blood samples were drawn for serum chemistries and 24-hour urine collected. In addition, information on age at first appearance of clinical symptoms of ADPKD, age at diagnosis of hypertension, intracranial aneurysm, rupture of intracranial aneurysm, and renal failure was recorded.

Information from 1112 participants, 648 women (58.3%) and 464 men (41.7%), has been mapped to the CDISC SDTM standard. There were 165 deaths and 342 ESRD events with timing information. Additional descriptive statistics on this population are presented in Figure 7 through Figure 22.

Summary of University of Colorado Clinical Protocol for Collection of Data

Subjects were evaluated during a two-day admission to the General Clinical Research Center at the University of Colorado Hospital or to the Pediatric Research Center at the Children's Hospital.

Each subject had the following standardized testing performed and all information was recorded in a standardized format:

1. Standard history performed by one of the study clinical investigators or nurse practitioner

2. Standard physical examination performed by one of the study investigators or nurse practitioner. BP recorded by a trained nurse- with measurements performed in both sitting and supine positions minimum of three measurements for each. Hypertension was defined as BP > 140/90 mm Hg, systolic/diastolic or taking antihypertensive medications.

3. Blood was collected for serum and plasma chemistries including, sodium, potassium, chloride, urea nitrogen, creatinine, carbon dioxide, uric acid, albumin, calcium, magnesium, phosphorous, alkaline phosphatase, AST, GGT, and bilirubin. In addition, blood hematology included full and differential blood counts. Creatinine measures before 3/29/2009 were not by IDMS standardized methodology; there are no creatinine data on or after 3/29/2009. All analyses were performed in the hospital laboratory. In addition, a blood sample was obtained for genetic analysis (PKD1 or PKD2 determination by linkage analysis).

4. Two 24-hour urines were collected for creatinine and protein analysis, sodium, potassium, urea, uric acid, calcium, magnesium and phosphate. Creatinine clearance was calculated by the MDRD equation and reported as the mean value for two, 24 hour urines. All analyses were performed in the hospital laboratory.

5. US for kidney and liver volume and determination and cyst counts. Cyst counts were recorded categorically as 0 (0 cysts), 1 = < 5 cysts, 2 = 6-15 cysts and 3 = > 15 cysts (details

of imaging are provided in imaging protocol). Radiology measurements were recorded in a standardized format.

6. A diagnosis of ADPKD was made for those subjects meeting Ravines criteria {Ravine 1994}

7. Longitudinal data concerning date of onset of ESRD was collected on subjects. Date at onset of ESRD was defined as date of initiation of dialysis or date of transplant.

4.1.2   Mayo Clinic Database

Approximately 2,871 patients with ADPKD seen at the Mayo Clinic since 1984 have data contained in the database. The Mayo PKD database was initially approved by the Mayo IRB on August 19th, 1997 (IRB #1121-97). On October 18th, 2001, the IRB approval for the database was replaced by IRB PR285-00, which is linked to the NIH grant "Cystic Kidney Disease: Disease Spectrum and Genotype-Phenotype Correlations." PR285-00-03 was last approved by the IRB on August 22nd, 2012. Data include patient demographics, death information, laboratory test results, results of imaging studies, clinical information, and family history.

The Mayo Clinic has supplied CDISC STDM mapped data on 1010 participants including 607 women (60.1%) and 403 men (39.9%). There were 68 deaths and 198 ESRD events with timing information. Additional descriptive statistics can be found in Figure 7 through Figure 22.

Summary of Mayo Clinic Clinical Protocol for Collection of Data

Subjects are evaluated during a Nephrology/General Medicine consultation at Mayo Clinic Rochester in Minnesota.

Subjects have the following testing performed and all information is recorded in the medical record:

1. Medical history including present illness and systems review, past medical/surgical history, social and family history performed by one of the PKD group specialist or other designated Mayo Clinic consultant

2. Physical examination performed by one of the PKD group specialists or other designated Mayo Clinic consultant. Vital signs such as temperature, pulse, respirations, and BP, and anthropometric data (height, weight, body surface area and BMI) recorded by a clinical assistant. Hypertension is defined as BP > 140/90 mm Hg, systolic/diastolic or taking antihypertensive medications.

3. Blood samples are collected for serum and plasma chemistries including, but not limited to, sodium, potassium, calcium, phosphorus, total protein, glucose, creatinine, urea nitrogen, albumin, lipid profile, liver enzymes, and bilirubin. In addition, blood hematology, including full and differential blood counts. Creatinine measures before October 18th, 2006 were not performed using IDMS standardized methodology. Creatinine measures on or after October 18th, 2006 were performed using IDMS standardized methodology. Blood analyses are performed at Mayo central clinical laboratory or the patient may provide a report from an

accredited laboratory. In addition, a blood sample for genetic analysis (PKD1 or PKD2 mutation screen) may be obtained.

4. Twenty-four-hour urine samples are collected for routine urinalysis, including creatinine and protein analysis, sodium, potassium, urea, uric acid, calcium, magnesium and phosphate. eGFR is calculated by the MDRD equation appropriate for the creatinine measurement technique. All analyses are performed at Mayo central clinical laboratory or the patient may provide a report from an accredited laboratory.

5. Imaging studies for diagnosis of the disease, evaluation of disease severity/progression, or diagnosis/assessment of renal and extrarenal complications are acquired following each patient appropriate recommendation. Ultrasonography may have been used as an initial diagnostic modality, followed by abdominal CT or MRI depending on each case. Kidney and liver volume measurements are performed on MRI or CT. Radiology measurements are recorded in a standardized format.

6. A diagnosis of ADPKD was made in those subjects meeting Ravine's criteria, or if the patient possesses a PKD1 or PKD2 mutation identified by sequence analysis or genetic diagnosis based on linkage analysis. A presumptive diagnosis is made in the absence of a family history of ADPKD, when the patient has more than ten cysts per kidney in the absence of manifestations suggestive of a different renal cystic disease.

7. Longitudinal data concerning date of onset of ESRD or patient's death is collected and registered in the patient's medical record. Date at onset of ESRD is defined as date of initiation of dialysis or date of kidney transplant.

8. The Mayo PKD database is populated electronically with Mayo central clinical laboratory data, or abstracted when necessary.

4.1.3   Emory University Database

Between 1998 and present day, patients have been recruited for ongoing natural history and genetic studies of ADPKD at Emory University. Dr. Chapman had previously worked with the University of Colorado and the format for this study in many ways reflects the observational registry performed at the University of Colorado. The Emory University database population consists of over 700 carefully phenotyped individuals from approximately 400 families participating in a longitudinal observational program supported by the Polycystic Kidney Disease Foundation (COHORT Study).

Information from 376 participants, 229 women (60.9%) and 147 men (39.1%), has been mapped to the CDISC SDTM standard. There were eight deaths and 121 ESRD events with timing information. Additional descriptive statistics can be found in Figure 7 through Figure 22.

Summary of Emory University Clinical Protocol for Collection of Data

One or more affected members of each family are examined during a two-day visit at the Emory University General Clinical Research Center (now Atlanta Clinical and Translational Science Institute Clinical Research Network), at which time medical and family history is obtained and complete physical examinations are performed, including BP measurements determined 48 times over a 24-hour time

period. Blood samples are drawn for serum chemistries and 24-hour urines are collected. In addition, information on age at first appearance of clinical symptoms of ADPKD, age at diagnosis of ADPKD, of hypertension, the presence of intracranial aneurysm or rupture of intracranial aneurysm, and ESRD is recorded. Individuals are tracked annually in standardized fashion with clinical information including height, weight, 50 blood pressure measurements/day, I^{125} iothalamate clearance determinations, and serum creatinine determinations. DNA from each family is available. Extensive renal imaging data, including MR images, US images, and CT images have been obtained. Extensive questionnaires regarding birth-weight, dietary intake, over-the-counter and prescription medication use, dosage, and duration of use are collected. Significant dietary exposure measures, including the Willett semi-quantitative food questionnaire, 24-hour and three-day dietary recalls, and 24-hour measures of urinary urea, sodium, and potassium excretion are included. Data from the COHORT study are maintained in a relational database in Access that allows for query reports that link subject with imaging, biochemical, and historical data. This is kept in a secure HIPPA-compliant environment. Quality assurance is maintained with regular validation of 10% of the data entries. An extensive data dictionary defining all variables is kept on site. IRB approval has been continuous since 1998.

Each subject has the following standardized testing performed and all information is recorded in a standardized format:

1. Standard history performed by one of the study clinical investigators or co-investigators

2. Standard physical examination performed by one of the study investigators or co-investigator. Blood pressure and heart rates are recorded by a trained nurse. Measurements performed in the sitting positions every 30 minutes over 24 hours. Hypertension is defined as an average 24-hour BP > 140/90 mm Hg, systolic/diastolic or taking antihypertensive medications.

3. Blood is collected for serum and plasma chemistries including, sodium, potassium, chloride, urea nitrogen, creatinine, carbon dioxide, uric acid, albumin, calcium, magnesium, phosphorous, alkaline phosphatase, AST, GGT, and bilirubin. In addition, blood hematology includes full and differential blood counts. Creatinine measures before April 8th, 2009 were not performed using IDMS standardized methodology. Creatinine measures on or after April 8th, 2009 were performed using IDMS standardized methodology. All analyses are performed in the hospital laboratory. In addition, a blood sample was obtained for genetic analysis (PKD1 or PKD2).

4. Two 24-hour urines are collected for urinary creatinine, protein, sodium, potassium, urea excretion determinations. Measured I^{125} iothalamate clearances are determined for GFR determinations. eGFR is calculated by the MDRD equation appropriate for the creatinine measurement technique.

5. MRI for TKV. Radiology measurements are recorded in a standardized format

6. US for kidney volume. Radiology measurements are recorded in a standardized format.

7. Longitudinal data concerning date of onset of ESRD is collected on subjects. Date at onset of ESRD is defined as date of initiation of dialysis or date of transplant.

4.1.4   CRISP I /II (NCT01039987)

CRISP I is a multicenter, prospective, longitudinal study of the natural history of ADPKD to determine if MRI can detect small changes in renal structural involvement over a short period of time in ADPKD. There were 241 subjects from four sites including Kansas University Medical Center, Emory University, the Mayo Clinic, and University of Alabama at Birmingham. The primary outcome variables measured were changes in TKV, TCV and % cystic involvement assessed by MRI over time. These were measured in ADPKD individuals with relatively normal kidney function but with two-thirds having characteristics consistent with risk for progression to renal failure. This study also assessed the use of US to approximate the measurements made by MRI with the estimation of % cross-sectional kidney cystic involvement. The relationship between these structural changes and changes in kidney function, risk stratification for progression to ESRD, and clinical events in ADPKD were also determined.

The goal of the CRISP II Study is to continue to observe individuals from CRISP I in a prospective fashion, to evaluate the power and reliability of MRI to predict disease progression in ADPKD including a change in both TKV and kidney function over time, extending the preliminary observations of CRISP I. CRISP II has sought to ascertain the extent to which TKV TCV and liver cyst volume or qualitative measures of kidney cyst distribution and character predict kidney function decline. This study also sought to develop other novel biomarkers of disease severity in ADPKD. CRISP II extended the preliminary observations of 202 CRISP I subjects to ascertain the extent to which age- and sex-adjusted measurements of renal blood flow by MR technology predict the rate of increase in TKV; and how renal blood flow and TKV predict the rate of kidney function decline in ADPKD.

All data from both CRISP I and II were converted to a CDISC SDTM structure. There were no deaths or ESRD events during CRISP I. In CRISP II there were two deaths and eight ESRD events with data available regarding the start of ESRD. Additional descriptive statistics can be found in Figure 7 through Figure 22.

Summary of CRISP I Clinical Protocol for Collection of Data

Cohorts of male and female patients who had ADPKD between 15 to 46 years of age were followed annually for four total visits. Eligibility required an ADPKD diagnosis by the criteria of Ravine {Ravine 1994) and a measured or estimated (Cockcroft-Gault equation) creatinine clearance >70 ml/min/1.73m^2. Eligibility also required a sCr ≤1.6 mg/dl in men and ≤1.4 mg/dl in women. Patients were ineligible when they had other medical conditions besides hypertension that could affect kidney function (e.g., diabetes), if they had previous kidney surgery or if they could not undergo MR imaging. Two thirds of patients were required to have a diagnosis of hypertension or documentation of the presence of detectable proteinuria (> 300 mg/day). These criteria defined a cohort of ADPKD participants who were early in the course of their disease but at risk for progression to ESRD. The remaining participants were at low risk for progression to renal failure but were similar in age, race, and gender distribution as compared to those at high risk for progression to ESRD.

Participants were recruited based on their risk for progression to ESRD in a 2:1 ratio of high:low risk individuals.

High risk

- Hypertension diagnosed prior to age 35 years

- Gross hematuria in males with ADPKD prior to age 30 years

- Greater than 300 mg urinary protein excretion in 24 hours in an adequate urine collection, defined as > 15 mg/kg/day and < 25 mg/kg/day creatinine excretion

- Diagnosis of ADPKD in-utero or in the first year of life

Low Risk

- Absence of all risk factors

Exclusion Criteria at screening

1. Age at the time of enrollment: less than 15 years

2. Age at the time of enrollment greater than 45 years

3. sCr concentration greater than 1.4 mg/dl in ADPKD women

4. sCr concentration greater than 1.6 mg/dl in ADPKD men

5. Weight greater than 350 lbs

6. Previous partial or total nephrectomy

7. Congenital absence of one kidney

8. Previous kidney cyst reduction performed percutaneously, laparoscopically, or surgically

9. Documented presence of renal vascular disease

10. Presence of indwelling ureteric stents

11. Greater than 2.0 grams of urinary protein excretion in a 24-hour period

12. Kidney parenchymal infection within six months of entry into study

13. Recent (less than six months) hospitalization for an acute illness (not including elective admissions)

14. Recent (less than six months) myocardial infarction or cerebral vascular accident (including transient ischemic attack, cerebral infarction, subarachnoid hemorrhage, or intracranial aneurysm rupture)

15. Current unstable angina

16. Diagnosis of non-insulin dependent or insulin dependent diabetes mellitus

17. Presence of systemic illness with kidney involvement (systemic lupus erythematosis)

18. ADPKD women who are pregnant, currently lactating or less than six months after delivery of a child

19. Significant pulmonary, cardiac, or liver disease not including polycystic liver disease

20. History or presence of malignancy

21. Significant anemia (hemoglobin less than 10 mg/dl)

22. Significant thrombocytopenia (Platelet count less than 70,000)

23. Significant neutropenia (Absolute Neutrophil Count less than 500)

24. Current psychological makeup of the potential subject that in the discretion of the principal investigator indicates that the subject will not successfully complete the study (e.g., psychiatric illness precluding informed consent, unable to maintain compliance with study visits)

25. Unable to provide written informed consent

26. Presence of MR incompatible clips

27. Previous clipping for intracranial aneurysm

28. Presence of cardiac pacemaker

29. Known claustrophobia to MR scanners

Assessments

Enrolled subjects were instructed to continue their current medications and to discontinue NSAID intake for at least seven days prior to evaluation. Subjects were not to initiate diuretic therapy within 14 days of evaluation. During the day prior to admission for baseline evaluation, subjects collected a 24-hour urine sample for the determination of urinary creatinine, sodium, potassium, urea, and albumin excretions. Microalbuminuria was defined as >30 mg/day urinary albumin excretion. All subjects underwent several determinations using standardized methods. Weight and height were determined at the time of admission. Blood pressures were measured in the left and right arm in the morning, prior to antihypertensive medication intake, after being seated for at least five minutes on three occasions three minutes apart using an oscillometric measuring device. Subjects then stood for five minutes and their blood pressure was measured three times in the arm that demonstrated the highest reading in the seated position. Hypertension was defined by current use of antihypertensive medications or a systolic and diastolic BP $\geq 140/90$ mmHg on multiple occasions.

All subjects underwent a standardized MRI protocol developed by the Imaging Committee and approved by the Steering Committee. Subjects were studied in the morning prior to medication intake and breakfast.

MR Images

MR images were obtained at each clinical site by a trained MR technologist or imaging specialist. Although all MR scanners were 1.5 T scanners, scanners at each site were either of a different model or manufacturer. The University of Alabama at Birmingham used GE LXI (General Electric Medical Systems, Milwaukee, WI, USA), Emory University used Gyroscan (Philips Medical Systems, Best-Leiden, The Netherlands), the Mayo Foundation used GE Sigma (General Electric Medical Systems, Milwaukee, WI, USA), and the University of Kansas Medical Center used Magnetron Vision Plus

(Siemens Medical Systems, Iselin, NJ, USA). Prior to each study, the MR scanner was adjusted for proper shimming. A 20-gauge intravenous angiocatheter was placed and infused with normal saline solution to maintain venous patency during the course of imaging. Electrocardiographic (ECG) pads were placed for ECG gating. Subjects were placed supine on the MR table with their arms either at their side or over their shoulders. A phased-array surface coil was positioned with its center over the inferior costal margin, estimated as the upper margin of the kidney. During this time, breath-holding instructions were given to the subjects. Scout scans were performed to locate the scan range of the entire kidney. Axial images were obtained that covered the most anterocaudal and posterocranial aspects of the kidneys. The field of view was maintained between 30 and 35 cm. Breath-held coronal T2-weighted images [single shot fast spin echo/half-Fourier acquired single turbo spinecho (SSFSE/HASTE)] with fat saturation were obtained with 3 mm and 9 mm fixed slice thickness and an image adjusted for a slice thickness between 3 and 9 mm in order to cover the kidneys with a single breath-hold. Neighboring image groups were overlapped by the designated slice thickness for each image acquisition. After the acquisition of T2-weighted images, three-dimensional volumetric interpolated breath-hold examination/fast multi-slice spoiled gradient echo (VIBE/FMPSPGR) coronal T1-wighted images without fat saturation were obtained with 3 mm fixed slice thickness. Following this, gadolinium was injected at 1 mL/second for a total of 20 to 30 mL or 0.1 mmol/kg. T1-weighted images were repeated with 3 mm slice thickness 120 and 180 seconds after initiation of the gadolinium injection. Following these image acquisitions, subjects were removed from the MR table and returned to the research clinic, for completion of the remaining studies.

GFR studies: Nonisotopic iothalamate renal clearances

Subjects were instructed not to eat food after midnight but to continue oral hydration. Subjects ingested no less than 600 mL of water prior to obtaining background iothalamate samples. After oral hydration, a blank urine and plasma sample was collected prior to injection of iothalamate meglumine (Conray 60%). Iothalamate (300 mg) was injected subcutaneously followed by a 60-minute equilibration period. Blood and urine samples were then collected within five minutes of each other, and bladder emptying was monitored by US. The urine sample from this collection was discarded and the time of voiding was recorded. After 45 minutes, blood and urine samples were collected with the times and urine volume recorded. Bladder emptying was monitored by ultrasound. The studies were considered satisfactorily completed if ultrasound monitoring of the bladder demonstrated <20 mL or <10% residual urine volume. Urine and plasma samples were immediately centrifuged, aliquotted, and packaged for shipping to the Mayo Medical Laboratories for measurement of iothalamate concentration.

Additional assessments

Blood was obtained prior to GFR studies for the determination of serum electrolyte, liver enzyme, hemoglobin, and total cholesterol concentrations and platelet and white cell counts. Blood was obtained from all participating African Americans to screen for the presence of sickle cell trait or disease or thalassemia using hemoglobin electrophoresis. All samples were collected into pre-chilled tubes and placed on ice before being centrifuged and separated and frozen at -80 degrees C until assayed.

During the baseline visit, patients underwent a focused history and examination. MRI was used to determine the TCV and TKV. The day before the baseline visit, a 24-hour urine sample was collected to determine the urine albumin to creatinine ratio (ACR).

Kidney function was measured by each method at baseline (2001) and at the follow-up visits in 2002, 2003, and 2004. After oral hydration, patients received a subcutaneous injection of nonradiolabeled iothalamate. After a 60-minute equilibrium period, each patient voided and the first plasma sample was drawn. After a timed 45- to 60-minute collection period to determine urine flow (V), a voided urine sample and a second plasma sample were obtained. Postvoid residuals were assessed by ultrasound after each void. The two plasma (P) samples and one urine (U) sample were assayed for iothalamate via capillary electrophoresis at the Mayo Clinic. Iothalamate concentrations in the plasma samples were averaged, and GFR was determined using the clearance equation ($U_{Iothalamate}V/P_{Iothalamate}$).

Serum was collected at each visit and assayed for creatinine at each site. All sCr levels were adjusted for calibration bias with the Cleveland Clinic laboratory used for deriving the MDRD equation.

Summary of CRISP II Clinical Protocol for Collection of Data

Eligibility and patient recruitment for CRISP II

CRISP I participants were invited to participate in CRISP II. At entry into CRISP II, participants met a number of inclusion and exclusion criteria. Exclusion criteria for participation in CRISP II were:

1. Current psychiatric or addiction or non-compliance disorder that in the discretion of the principal investigator indicates that the subject will not successfully complete the study

2. Current medical problem that in the discretion of the principal investigator would make unsafe the participation in the study

3. Unable to provide written informed consent

Participating clinical center (PCC) visits and annual blood samplings for participants who were pregnant were postponed until six months following the delivery of a child and termination of lactation. CRISP I participants with new MRI incompatible clips or pacemakers or who had developed severe claustrophobia were eligible for recruitment into CRISP II, but would not undergo MRI. To enroll in CRISP II, individuals provided written informed consent meeting the requirements of the local IRBs, which included at least two consent forms, one that covered the basic elements of the CRISP II study and a separate consent form requesting permission to contact family members. Consenting to the latter was not required to participate in the study. Separate consent forms were developed to obtain historical and clinical information and a blood sample from known affected family members and for site-specific studies not covered in the main study consent form.

The CRISP II protocol did not exclude participants enrolled in other interventional trials. CRISP II participants recruited into an interventional trial (e.g., HALT clinical trial) that required imaging had their visits for both trials coordinated to avoid duplication of tests and undue burden on the participant. Only data from baseline visits in interventional trials were used for CRISP II analyses.

Study Visits

Study visits included PCC visits on years 1 and 3; annual visits on years 2 and 4 to either the PCC or a local physician's office/laboratory; semi-annual telephone interviews; recruitment of family members, sample collection and DNA isolation.

PCC visits (years 1 and 3):

Participants were admitted to the in-patient General Clinical Research Center or the Clinical Research Network in the late afternoon or evening or in the morning prior to eating or taking medication. On admission, participants signed consent and underwent a formalized medical history interview. Information regarding medications (prescribed and over the counter), quality of life, and level and quality of pain were obtained using procedures identical to those used in CRISP I. Quality of life (SF-36v2), pain, and family history questionnaires were also obtained. Subjects had a complete physical examination with standardized blood pressure determinations. A B-HCG qualitative urine test was performed on women of childbearing potential. Blood and urine samples were collected in the morning, prior to morning hydration or taking medications or food.

Blood was collected for:

1. Serum Creatinine – Serum samples were obtained in duplicate, one processed at the local lab and the other frozen and batch shipped to the Cleveland Clinic Laboratory.

2. Total Electrolyte Panel – Sodium, potassium, chloride, total CO_2 (at PCC).

3. Lipid Panel – Total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol (at PCC).

4. Twenty mL was collected in two SST tubes (tiger-top, 10 mL each) and 16 mL in two PST tubes (green/grey-top, 8 mL each). Samples were centrifuged (without decanting) and shipped refrigerated (on frozen cold packs) to the NIDDK Biosample Repository at Fisher Bioservices on the day of collection, where they were aliquotted into 1 mL tubes and archived.

Urine was collected for:

1. Urine albumin and creatinine (at PCC).

2. Freshly voided urine specimens were centrifuged in 50 ml polypropylene tubes at 500 g for five minutes as soon as possible, with volume, processing times, and voiding times noted (processing times less than 20-30 minutes from the time of acquisition). Tubes were kept in ice throughout this process. The bottom 250μL pellet (sometimes barely- or non-visible) was transferred with a 1.0 mL pipette to a 1.5 mL eppendorf tube previously prepared with 750 μL of TriReagent (Molecular Research Center, Inc., Cincinnati, OH), and inverted several times and put on ice prior to freezing at -80˚C for future RNA/DNA retrieval. The remaining urine sample was then transferred to 10 mL polypropylene Falcon culture tubes, stored in six 5 mL aliquots, and sent to the NIDDK Repository at Fisher Bioservices.

3. Urine samples for MCP-1 analysis were sent annually from the NIDDK Repository at Fisher Bioservices to KUMC.

All participants were instructed to drink three 8-oz. glasses of water between 9:00 p.m. and 10:00 p.m. on the evening before the testing and to remain fasting but free to drink water *ad lib*. They were asked to go to bed at 10:00 p.m. In the morning between 6:00 a.m. and 8:00 a.m. they were asked to drink six 8-oz. glasses of water in preparation for the iothalamate clearance determination which started at 8:00 a.m. GFR determinations were performed using the short non-radiolabeled iothalamate clearance with

standardized conditions and monitoring of bladder emptying using a bladder scan to maximize accuracy. The concentrations of iothalamate in plasma and urine were measured by capillary electrophoresis.

The plasma and urine samples were packaged in a "refrigeration specimen" transport box and mailed to Mayo Medical Laboratories. The measurements were performed at Mayo Medical Laboratories.

After completion of the GFR determination, the participants underwent an MR examination of the kidneys and liver and determination of renal blood flow.

Blood pressure measurements

A standardized method for obtaining BP was used {Chapman 2010, Torres 2012b}. These measurements were obtained at the time of the PCC visits, annually for local patients or only at the 2007 and 2009 visits for the rest. Blood pressures were determined in the morning prior to antihypertensive medication intake using automated or non-automated oscillometric techniques (Dinemap, Critikon) and devices maintained and calibrated at the GCRCs or PCCs. The non-dominant arm (in terms of handedness) was used to obtain BP readings unless there was a reproducible (on at least three consecutive measurements) difference in systolic BP of 20 mm Hg or more between arms. If there was a reproducible difference in systolic blood pressure of 20 mm Hg or more between both arms, the arm with the higher blood pressure was used. In all other cases, the non-dominant arm was used. Participants were instructed to abstain from smoking and consuming caffeine for 30 minutes prior to taking their BP measurements. After sitting quietly for at least five minutes with the arm resting at heart level, three readings were obtained at least 30 seconds apart. If there was a difference of more than 10 mm Hg (systolic or diastolic) between the second and third readings in one sitting, a fourth and fifth reading were recorded for that sitting.

Serum creatinine measurements

sCr was determined annually for all participants. Blood was drawn at the PCC and serum samples were obtained in duplicate. One sample was for serum creatinine determinations at the PCC. The other was batch shipped every three months to the Cleveland Clinic for validation. Participants who later entered the HALT PKD study had their serum creatinine done at the annual HALT visit. For non-local participants who were unable to return to the PCC on years 2 and 4, a blood sample was obtained in duplicate at a local facility. For standardization purposes the local labs were contacted directly with the procedure to be followed.

MR Examination

The imaging protocol for CRISP II was revised from the MRI protocol used in CRISP I based on a Public Health Advisory issued by FDA on December 22nd, 2006 notifying healthcare professionals of 90 reports of Nephrogenic Systemic Fibrosis or Nephrogenic Fibrosing Dermopathy (NSF/NFD) in patients who had moderate to ESRD and received gadolinium-based contrast agents for MRI and MRA.

Although a causative relationship between gadolinium contrast medium and NSF/NFD has not been definitely established, published data raised the suspicion that there may be an association between NSF/NFD and gadolinium contrast medium in patients with compromised renal function. In view of these concerns, gadolinium contrast medium was not used in the revised MRI protocol.

Gadolinium-enhanced MRI facilitates the process of measuring the kidney volume and identifying the renal arteries, however, is not absolutely required. Instead, an additional fast imaging sequence, 2D true-FISP (FIESTA) without fat sat, was obtained to image the kidneys just as T2 imaging. This provided an additional cue to help delineate the kidney border on T1 images. In addition, 2D true-FISP (FIESTA) with fat sat was acquired to depict the renal arteries prior to the phase-contrast RBF measurement sequence. Bae and colleagues have examined the comparability of TKV measurements with and without gadolinium {Bae 2009}. Pre- and post-gadolinium 3D T1 (pre-T1, post-T1) MR images were obtained. The stereology method was applied to segment and measure kidney volumes. Kidney volumes obtained with and without gadolinium were highly correlated. Kidney volumes measured without gadolinium were slightly smaller than those with gadolinium, and percent differences between pre- and post-kidney volumes decreased with increasing kidney size {Bae 2009}.

MR images were obtained at each PCC using the procedures described below. After image acquisition, MR images were reviewed locally at each PCC site and securely transferred via internet connection to the Image Analysis Center (IAC).

The procedures for MR scanning of the heart (HALT study only), kidneys and liver were as follows:

Before each study, the MR scanner was adjusted for proper shimming.

1. Breath-holding instruction was provided, and the subject was coached prior to MR scanning. Administration of oxygen via nasal cannula could be used to improve the breath-hold capacity, particularly for subjects with limited breath-hold capacity.

2. EKG pads were placed over the chest. If EKG gating was not available or functioning, it could be replaced with a peripheral pulse gating.

3. The subject was placed supine on the MR table with his or her arms to the side.

4. A phased-array surface coil was positioned with its center over the inferior costal margin, i.e., over the expected location of the kidneys.

5. Scout scan was used to locate the scan range of the entire kidneys. A stack of axial images to cover the most anterocaudal and posterocranial aspects of the kidneys was highly recommended.

6. The field-of-view (FOV) was to be kept as small as possible (30-35 cm) without producing wrap-around artifacts.

7. Breath-hold, coronal T2 scan (SSFSE/HASTE with fat sat) with 9 mm fixed slice thickness, to be achieved in a single breath-hold if possible, were taken. Both kidneys were to be imaged completely without missing any anterior or posterior portions. This coverage assurance was critical for the following T1 imaging.

8. Coronal T1 scan (3D VIBE/FMPSPGR/LAVA without fat sat) with 3 mm fixed slice thickness (acquisition was performed at 6 mm thickness and then the slice was interpolated at 3 mm, i.e., in GE, ZIP =2 in the slice direction). The flip angle was kept at $\leq 15^{\circ}$. To improve SNR, the Bandwidth was kept low (62 kHz or 42 kHz) and/or the number of phase-encoding steps was increased. In GE LAVA sequence, turning off "optimize flip for CNR"

would allow for the change of the flip angle or bandwidth. Parallel imaging was not to be used (no SENSE, ASSET, iPAT, or GRAPPA).

9. Breath-hold coronal T2 scan (SSFSE/HASTE with fat sat) with 3 mm fixed slice thickness, requiring 1-4 breath-holds depending on the kidney size. As few breath-holds as possible were instructed to be used. The first scan should have covered the posterior aspect of the kidney. Neighboring image groups should have been overlapped by a single 3 mm slice.

10. Breath-hold coronal T2 scan (SSFSE/HASTE without fat sat) of the kidneys with adjusted slice thickness, 3-6 mm, i.e., the slice thickness best attainable with a single breath-hold was done. However, the adjusted slice thickness may not have remained the same in a follow-up MR scan if there was a change in the subject's breath-hold capacity or kidney size. The scan over the liver was repeated with the same slice thickness. This scan and the scan for the kidney should have shared one overlapping liver slice, i.e., the most posterior slice of the liver scan should be identical to the most anterior slice imaging the liver in the kidney scan. If more than two scans were required to cover the anterior liver, the neighboring scans were to be overlapped by one slice.

11. Breath-hold coronal 2D true-FISP (FIESTA) without fat sat with 3 mm fixed slice thickness, which required 1-2 breath-holds depending on the kidney size was done using as few breath-holds as possible. The first scan should have covered the posterior aspect of the kidney. Neighboring image groups were to be overlapped by a single 3 mm slice.

12. For renal blood flow measurement: Breath-hold, oblique-coronal 2D true-FISP (FIESTA) with fat sat with 4 mm fixed slice thickness at 2 mm spacing (i.e., overlap 50%) over the aorta and renal arteries was done. Typical parameters: 192x256 matrix, 75° flip angle, 125 kHz BW, 15-sec scan.

13. For renal blood flow measurement: Breath-hold, phase-contrast technique of renal blood flow measurement was done. From the FIESTA images, the renal arteries were identified. To accurately measure velocity, the imaging slice perpendicular to a vessel was chosen. Velocity encoding (VENC) value of 100 or 50 cm/sec was used. Small FOV (14-16 cm) and large matrix (256x192 or 512x512) were important for an accurate measurement of the vessel size. Segmented, prospectively cardiac-triggered phase contrast flow measurements were obtained to compute the mean and peak velocities, as well as the total mean flow, during the cardiac cycle.

For image transfers, images were pushed from the local PCC MR scanner to the PC workstation. For participant confidentiality, participant names and identifiers were removed and replaced with CRISP-ID numbers and accession numbers prior to image transmission to the IAC. A virtual private network (VPN) client was installed on the PC workstation to encrypt the data for secure transmission via the Internet. The IAC reviewed the images and generated quality control reports for PCCs. Images determined to be inadequate for measurement were reacquired.

The stereology method, a quantitative morphology by statistical analysis of the structures of random sections, is widely used in cytopathology and medical imaging analysis. A point-counting stereologic technique involves a simple, fast method of segmenting an object by counting the number of intersections of a randomly oriented and positioned grid over the object. This method does not require border tracing

or threshold determination, but relies on the operator's decision of selecting each point that intersects the object. The areas of the whole kidney in each image can be calculated from the collection of points, and volume measurements can be made from a set of contiguous images.

Analysis software, written by the Mayo Foundation, was utilized for making stereology measurements. Each volumetric measurement was made by a trained analyst at the IAC, and reviewed by a radiologist for quality control. Agreement between the radiologist and technician in the CRISP 1 Study was very high (97%). The result from the radiologist's review of stereology measurements was used to calculate the whole kidney volume {Sutters 2003}.

Annual fasting serum creatinine sample collections:

On off years, participants had blood samples collected either at the PCC or at their respective clinics for the determination of creatinine concentrations.

Semi-annual telephone interviews:

During the interviews, information regarding medication changes, hospitalizations, doctor visits, and outpatient procedures was recorded by study coordinators. A follow-up study form was completed after each telephone interview. Any physician who examined/treated the participant since the last visit or telephone interview was contacted to obtain information about the participant's health.

Recruitment of family members, sample collection, and DNA isolation:

A major component of CRISP II was to collect more comprehensive family histories of all CRISP I patients and draw an electronic pedigree for each family. Identified affected family members who agreed to participate were consented into the study and clinical and imaging data from the patient was retrieved from clinical records. A blood sample was collected for a determination of serum creatinine at the Cleveland Clinic laboratory (unless the participant was on dialysis or has received a transplant) and for DNA extraction and the establishment of EBV transferred lymphoblastoid cell-lines, employing the NIDDK Center for Genetic Studies, Rutgers University Cell and DNA Repository. Samples were sought from all traceable individuals from each of the families with proven ADPKD by established imaging criteria.

Participants were asked to complete a lifestyle questionnaire (to assess smoking history, caffeine exposure, estrogen exposure, and levels of physical activity) and a family history questionnaire to further extend the traceable family. When possible, the most recent CT or MR examination of the abdomen, or if not available, the most recent ultrasound images were reviewed and renal volume estimated using established formulae. TKV was calculated by the ellipsoid formula: Volume = length x width x thickness x pi/6, using maximum length in longitudinal plane and for width and thickness in the transverse plane perpendicular to the longitudinal axis of the kidney at the level of the hilum. If only coronal plane films were available, the kidney depth was assumed to be equal to the width of the hilum so that the formula becomes: Volume = length x (width) squared x pi/6. All of this clinical and lifestyle information, plus the available genetic information on the family, is stored in the CRISP database that is maintained by the Data Coordinating and Imaging Analysis Center at the University of Pittsburgh.

4.1.5   Additional Database Considerations

Common registry/study patients

Subjects from Emory and Mayo who subsequently participated in CRISP are known to the respective centers. A confidential link between the subject identifier at the registry and the subject identifier for CRISP was established to avoid double counting of individuals. There are 129 subjects (Mayo: 56, Emory: 73) who are captured in one of these patient registries who also participated in the CRISP study.

Adjudication process for PKD common subjects

Because it is possible that conflicting information was submitted for these common patients, a process was established to check and, if needed, reconcile the data. Based on analysis needs, we narrowed our review of the common subject data to following outcomes/covariates: death, hypertension, gross hematuria, urinary tract infections, kidney stones, hospitalization, diagnosis, and race. Conflicts for sex and date of birth had been previously resolved during the data conversion stage. For common data that showed conflicts, a Reconciliation Worksheet highlighting data differences was created. Adjudication rules were established for each set of data to help determine how conflicts should be resolved (see **Table 12**). All worksheets were reviewed by two physicians to approve the rules used and/or finalize all adjudication decisions. After all records were adjudicated, records were added to the appropriate Supplemental Qualifier dataset to indicate which data should be used.

Table 12: Common Subjects Adjudication Rules

Data	Adjudication Rules
Death	• If death dates differ by greater than one month perform a chart review to determine which date is of death is correct. • If death dates differ by less than or equal to one month, use the earliest death date.
End-Stage Renal Disease	• If ESRD dates differ by greater than one month perform a chart review to determine which date is of ESRD is correct. • If ESRD dates differ by less than or equal to one month, use the earliest ESRD date.
Hypertension	• If hypertension diagnosis dates differ by greater than three years perform a chart review to determine which date is of diagnosis is correct. • If hypertension diagnosis dates differ by less than or equal to three years, use the earliest diagnosis date.
Gross Hematuria	• If an event is reported with an event date, assume it is correct.
Urinary Tract Infection	• If an event is reported with an event date, assume it is correct.

Kidney Stones	• If an event is reported with an event date, assume it is correct.
Hospitalization	• Pull all records with inconsistent hospitalization dates.
Diagnosis	• Use known diagnosis over unknown diagnosis.
Race	• Use known race over unknown race.

4.2 Data Statistics and Plots

Several populations are referred to in this document. The following summarizes and defines the primary populations that are eligible for analysis:

Total Subjects with one or more image measurements ………………………………. 2355

    Subjects with two or more images (> six months apart) ……............................ 1182

    Subjects with one image (or > one image but < six months apart) …………..…… 1173

The following histograms are included to provide base data statistics for the populations that are eligible for analysis.

Figure 7: Distribution of Age at Study Entry (from Total Population; n=2355)
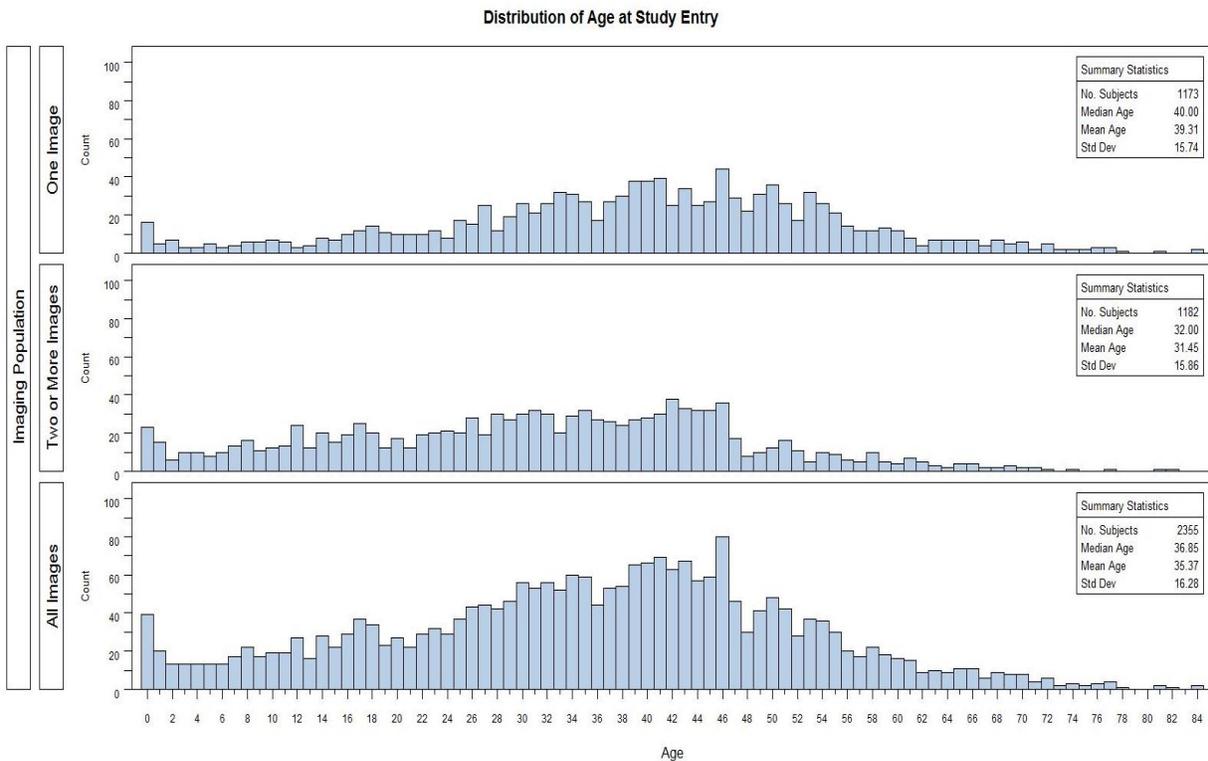
Figure 8: Distribution of Year of Study Entry (from Total Population; n=2355)
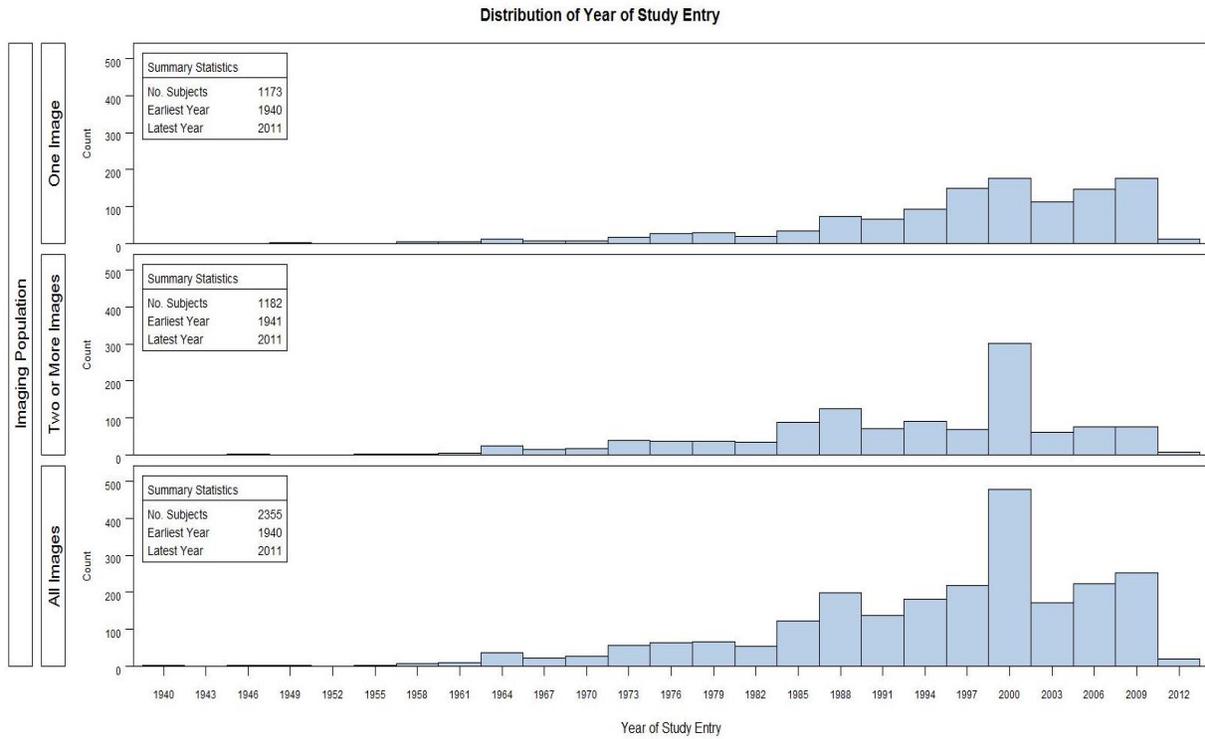
Figure 9: Distribution of Age at Death (All Deaths in Total Population; n=242)
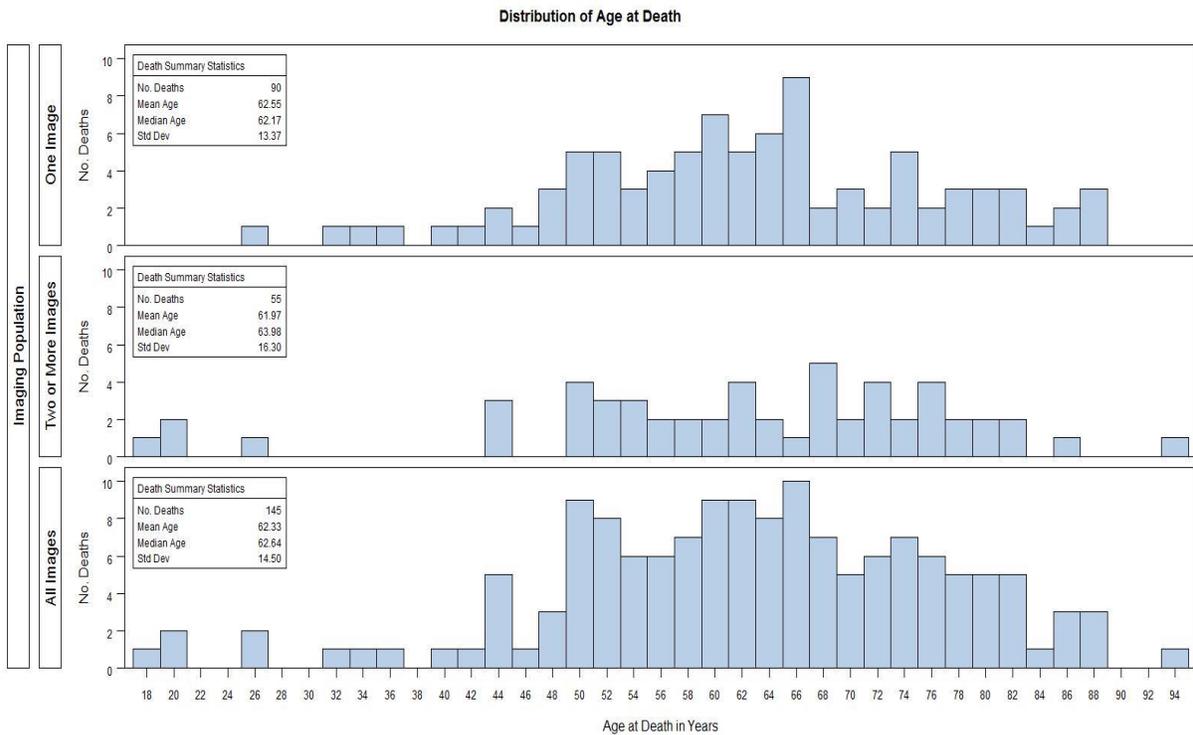
Figure 10: Distribution of Age at ESRD (All ESRD events in Total Population; n=668)
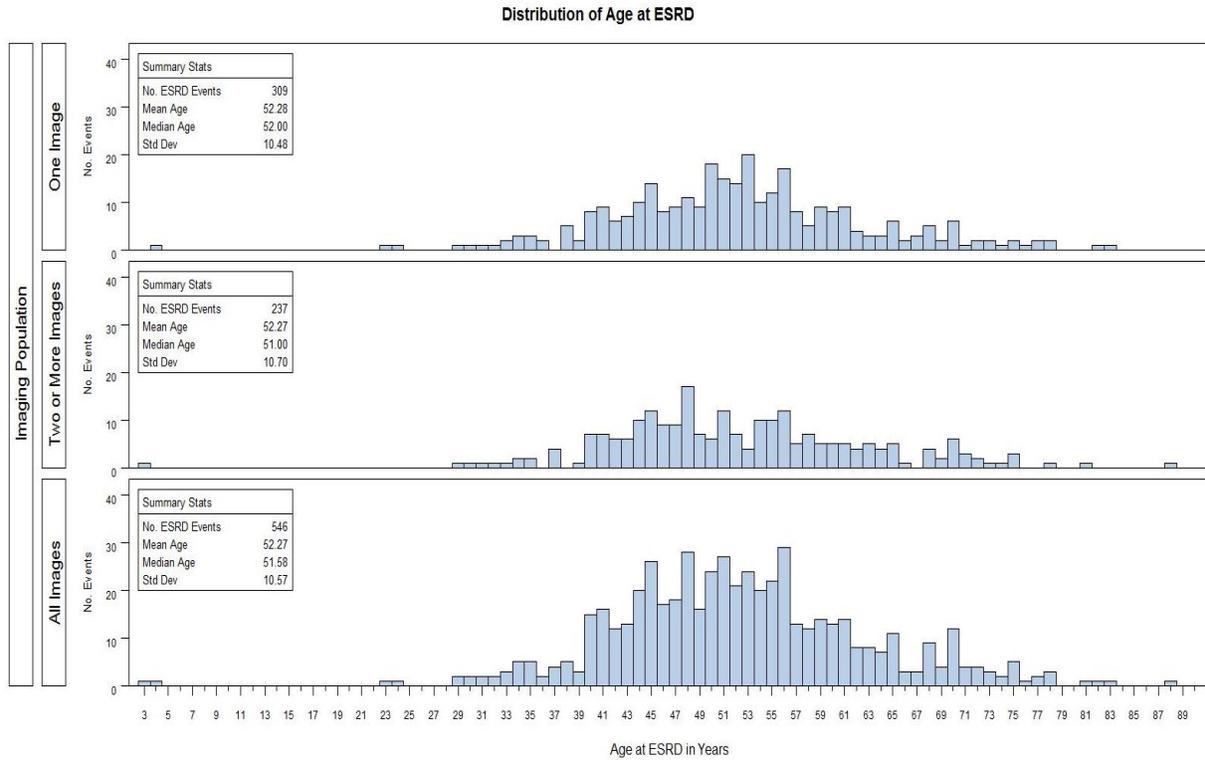
Figure 11: Distribution of Sex (from Total Population; n=2355)
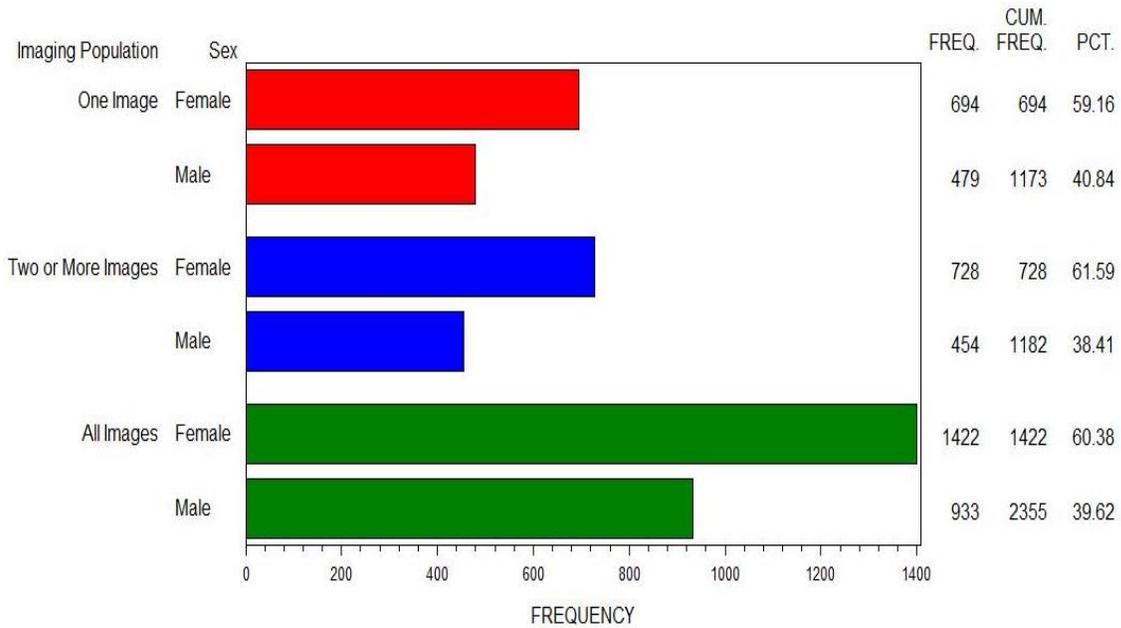
Figure 12: Distribution of Genetic Mutation (from Total Population; n=2355)
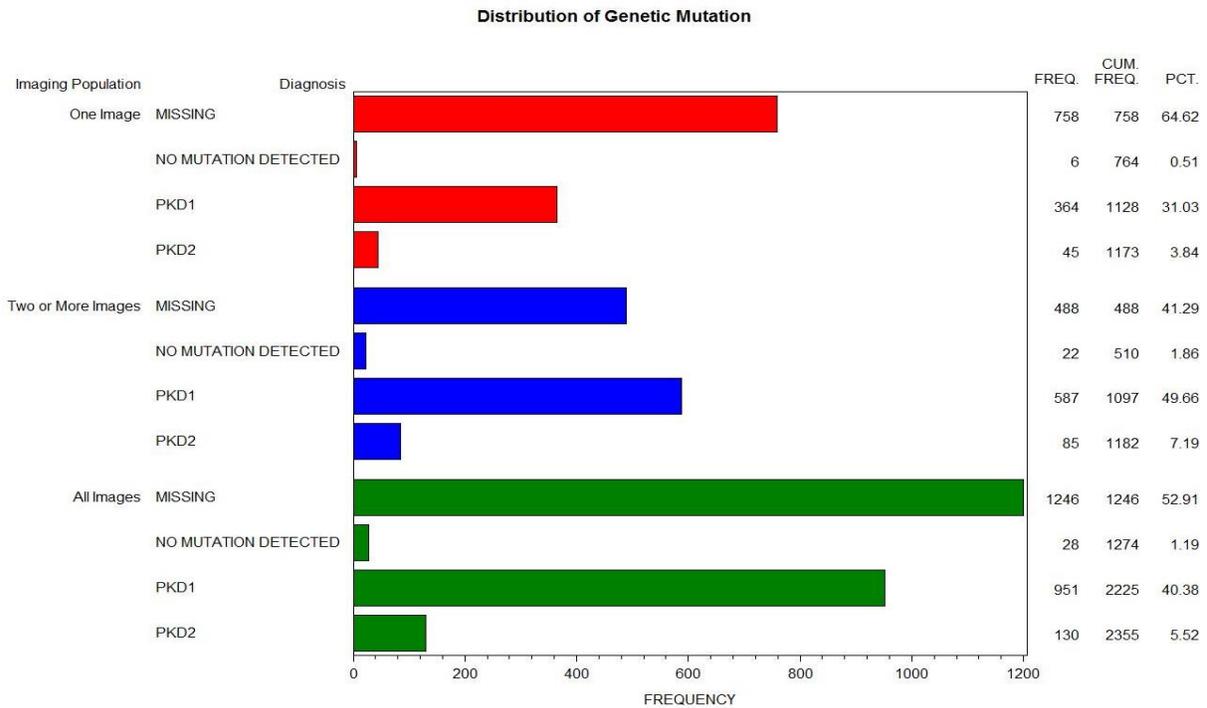
Figure 13: Distribution of Race (from Total Population; n=2355)
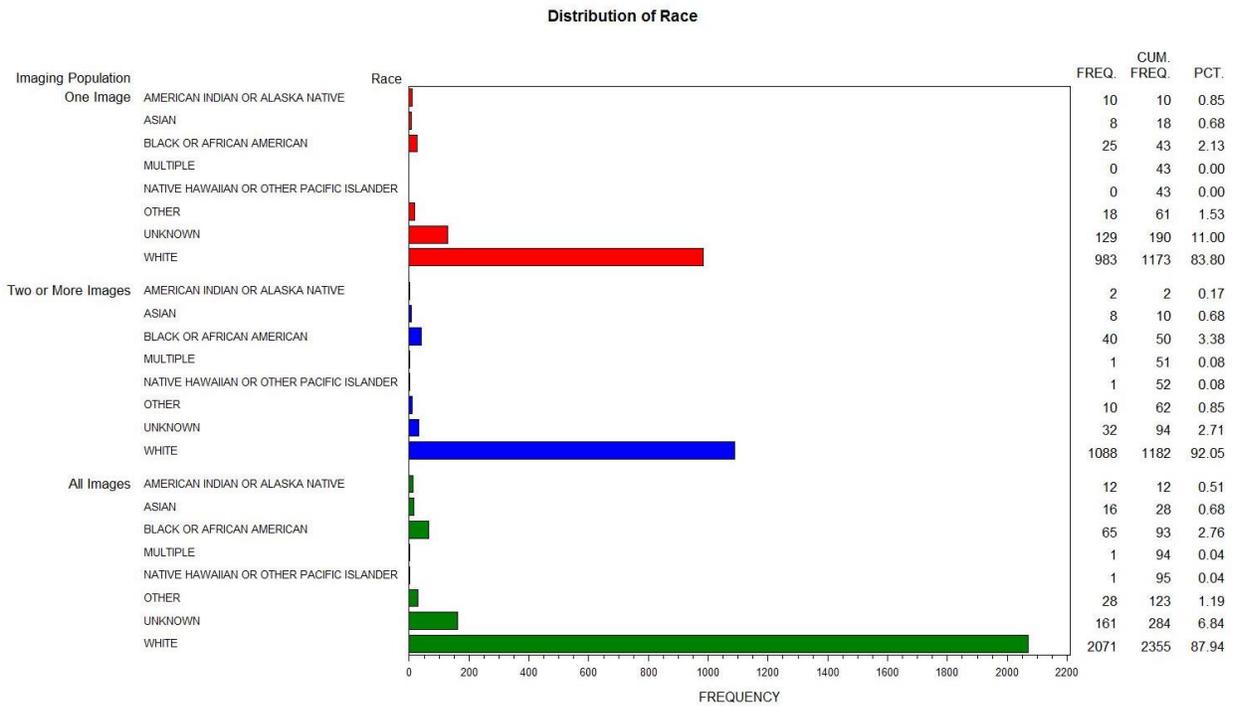
The following additional histograms are included to provide base data statistics for the populations of interest by site. Please note that for these 'by site' plots, Common Subjects are counted in both CRISP and the Registry site (either Emory or Mayo), but the duplicates are removed in the totals.

Figure 14: Distribution of Age at Study Entry (By site; from Total Population; n=2355)
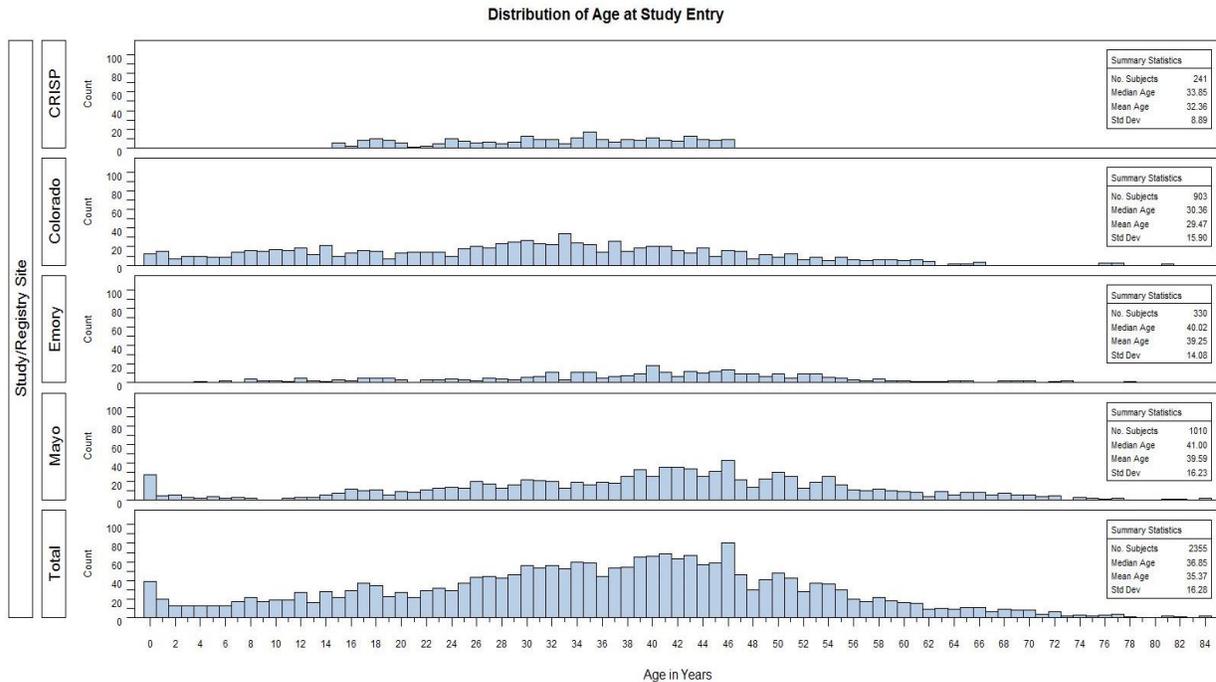
Figure 15: Distribution of Year of Study Entry (By site; from Total Population; n=2355)
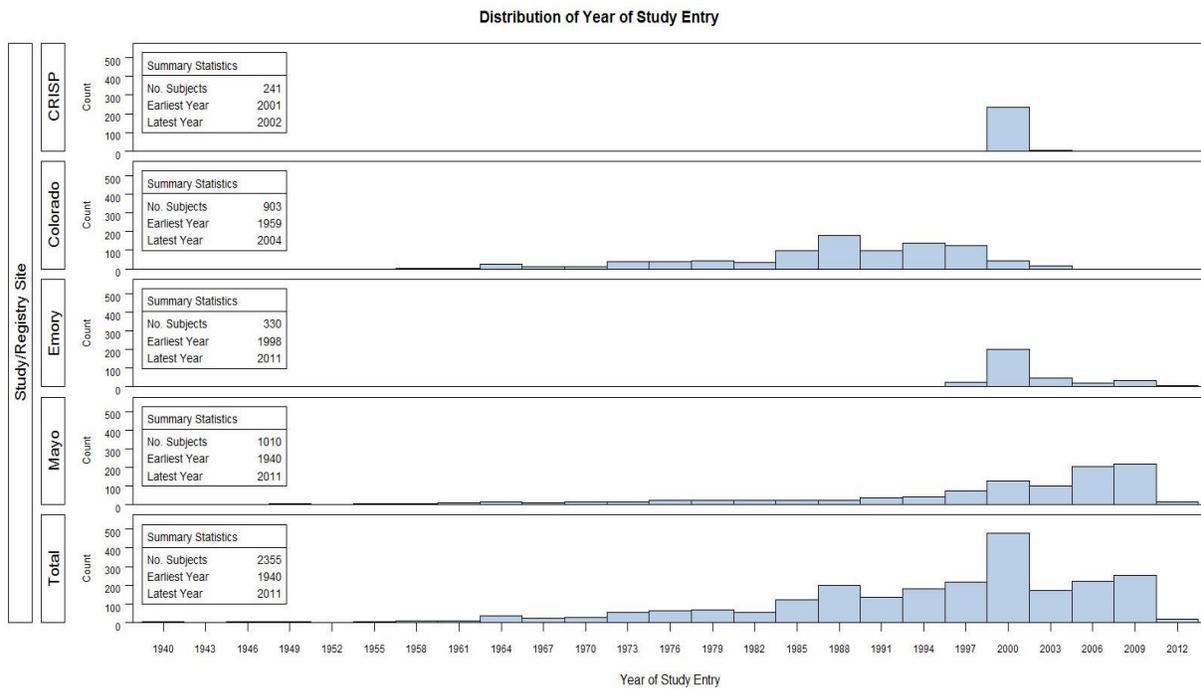
Figure 16: Distribution of Age at Death (By site; All Deaths in Total Population; n=242)
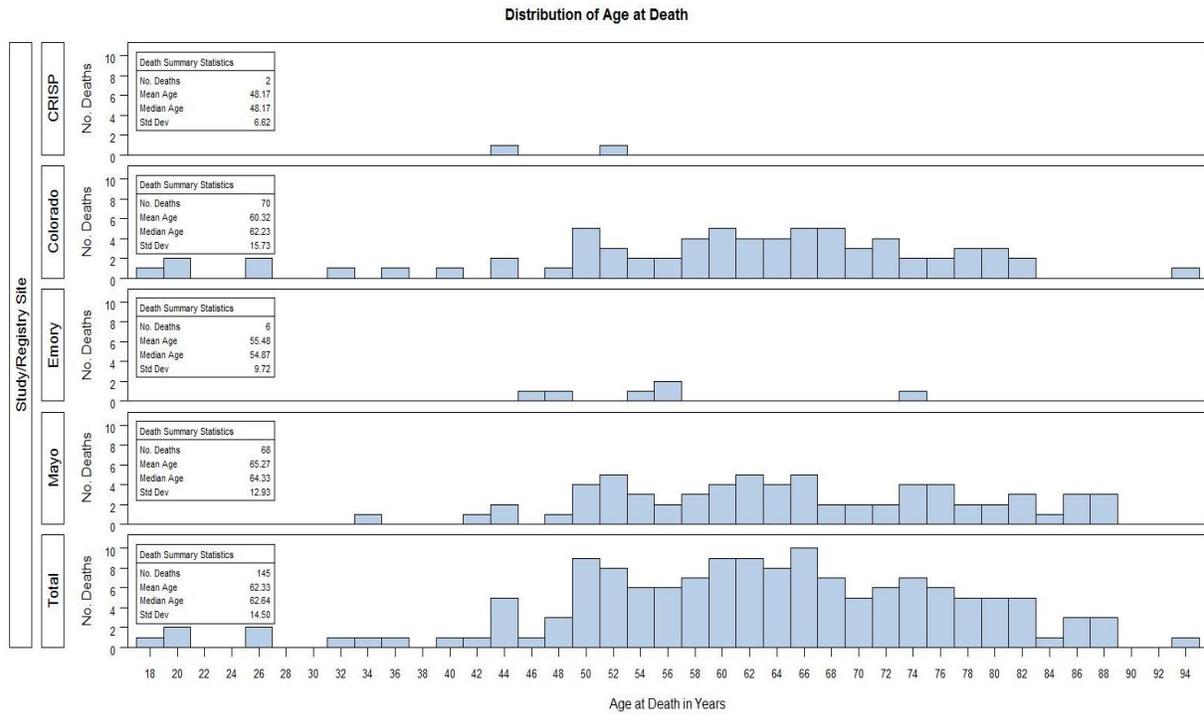
Figure 17: Distribution of Age at ESRD (By site; All ESRD events in Total Population; n=668)
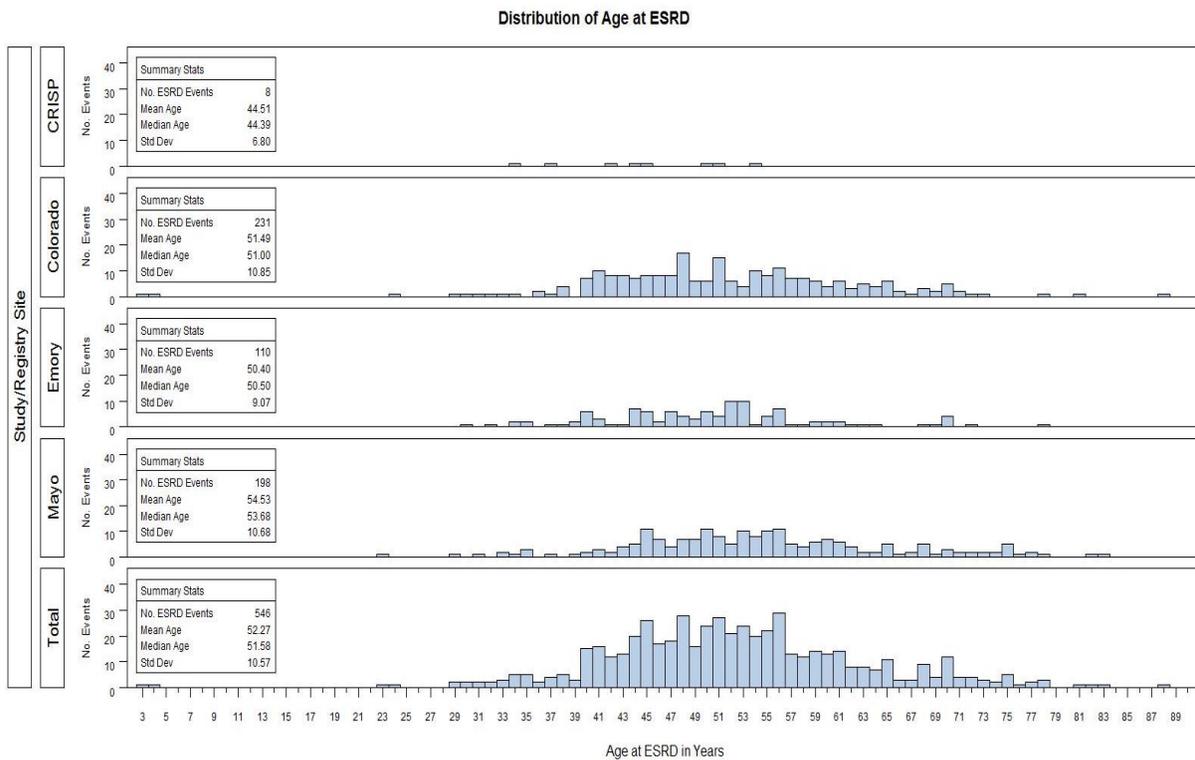
Figure 18: Distribution of eGFR at First Image (By site; All eGFR values at First Image; n= 1792)
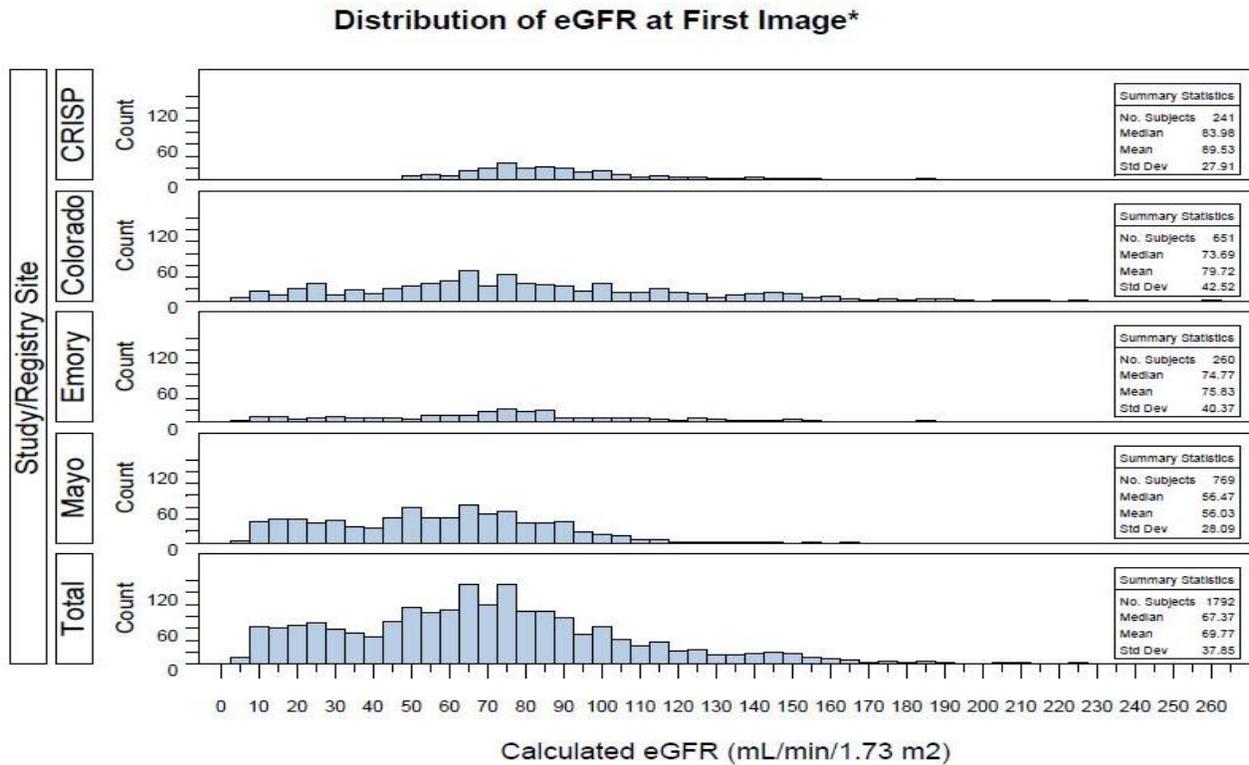
First image corresponds to the first image where a valid serum creatinine was measured on or after, and within 365 days of that image.

Figure 19: Distribution of Sex (By site; from Total Population; n=2355)
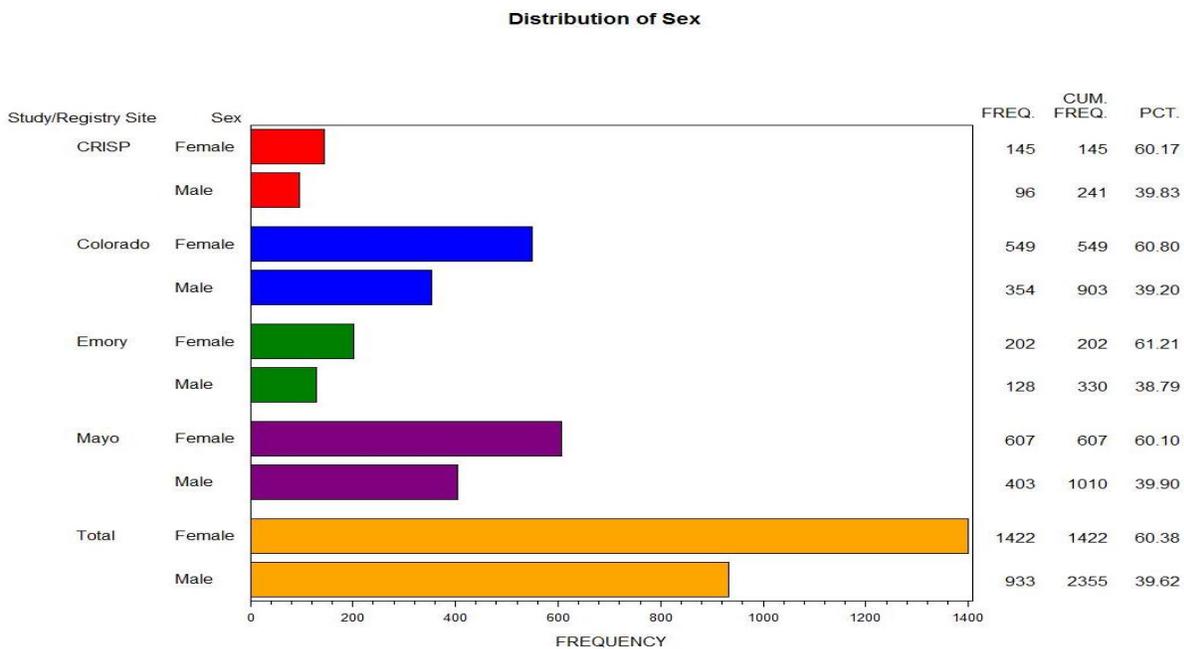
Figure 20: Distribution of Genetic Mutation (By site; from Total Population; n=2355)

Distribution of Genetic Mutation

Study/Registry Site	Diagnosis	FREQ.	CUM. FREQ.	PCT.
CRISP	MISSING	2	2	0.83
	NO MUTATION DETECTED	13	15	5.39
	PKD1	191	206	79.25
	PKD2	35	241	14.52
Colorado	MISSING	513	513	56.81
	NO MUTATION DETECTED	0	513	0.00
	PKD1	359	872	39.76
	PKD2	31	903	3.43
Emory	MISSING	76	76	23.03
	NO MUTATION DETECTED	0	76	0.00
	PKD1	231	307	70.00
	PKD2	23	330	6.97
Mayo	MISSING	655	655	64.85
	NO MUTATION DETECTED	18	673	1.78
	PKD1	278	951	27.52
	PKD2	59	1010	5.84
Total	MISSING	1246	1246	52.91
	NO MUTATION DETECTED	28	1274	1.19
	PKD1	951	2225	40.38
	PKD2	130	2355	5.52

FREQUENCY

Figure 21: Distribution of Race (By site; from Total Population; n=2355)

Distribution of Race

Registry Site	Race	FREQ.	CUM. FREQ.	PCT.
CRISP	AMERICAN INDIAN OR ALASKA NATIVE	0	0	0.00
	ASIAN	2	2	0.83
	BLACK OR AFRICAN AMERICAN	28	30	11.62
	MULTIPLE	1	31	0.41
	NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	0	31	0.00
	OTHER	1	32	0.41
	UNKNOWN	0	32	0.00
	WHITE	209	241	86.72
Colorado	AMERICAN INDIAN OR ALASKA NATIVE	7	7	0.78
	ASIAN	5	12	0.55
	BLACK OR AFRICAN AMERICAN	7	19	0.78
	MULTIPLE	0	19	0.00
	NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	0	19	0.00
	OTHER	11	30	1.22
	UNKNOWN	23	53	2.55
	WHITE	850	903	94.13
Emory	AMERICAN INDIAN OR ALASKA NATIVE	0	0	0.00
	ASIAN	3	3	0.91
	BLACK OR AFRICAN AMERICAN	34	37	10.30
	MULTIPLE	0	37	0.00
	NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	0	37	0.00
	OTHER	5	42	1.52
	UNKNOWN	5	47	1.52
	WHITE	283	330	85.76
Mayo	AMERICAN INDIAN OR ALASKA NATIVE	5	5	0.50
	ASIAN	7	12	0.69
	BLACK OR AFRICAN AMERICAN	12	24	1.19
	MULTIPLE	1	25	0.10
	NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	1	26	0.10
	OTHER	12	38	1.19
	UNKNOWN	133	171	13.17
	WHITE	839	1010	83.07
Total	AMERICAN INDIAN OR ALASKA NATIVE	12	12	0.51
	ASIAN	16	28	0.68
	BLACK OR AFRICAN AMERICAN	65	93	2.76
	MULTIPLE	1	94	0.04
	NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	1	95	0.04
	OTHER	28	123	1.19
	UNKNOWN	161	284	6.84
	WHITE	2071	2355	87.94

FREQUENCY

Figure 22: Number of Endpoints (By site; from Total Analysis Population)
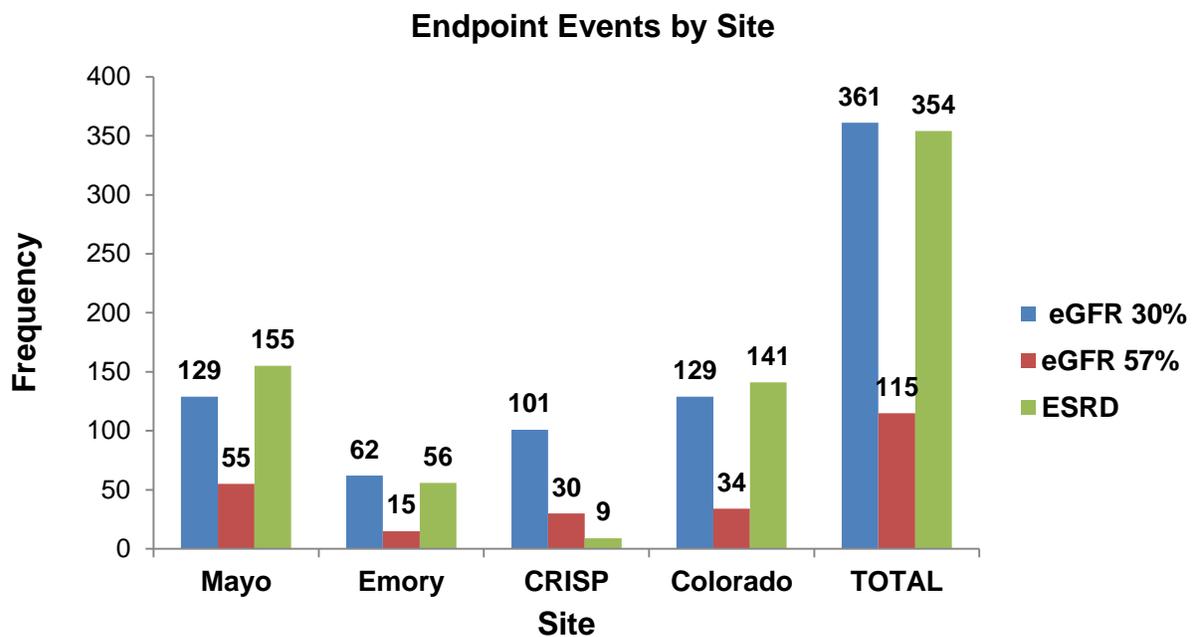
4.3   Total Kidney Volume Imaging Modalities

Medical imaging is gaining an important role in clinical trials. This has been driven by significant improvements in medical imaging technology and quality and the increasing need to leverage these technologies to reduce drug development time. The FDA's Critical Path Initiative acknowledges the potential value of imaging as a research tool in drug development {www.fda.gov/downloads/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/UCM077258.pdf}. In addition, the recent FDA Guidance for Industry on the Qualification of Drug Development Tools acknowledges that biomarkers may assess many different types of biological characteristics or parameters including radiographic or other imaging-based measurements {www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM230597.pdf}.

Several imaging modalities have been utilized to determine TKV in patients with ADPKD. These include US, CT and MRI.

Ultrasonography was the earliest method used to measure TKV *in vivo*, and has the advantage of being widely available, non-invasive, without radiation exposure, easily performed, and inexpensive. However, despite good correlation with kidney volume *ex-vivo* {Hricak 1983}, measurements of normal kidney volume *in vivo* have shown relatively poor accuracy and reliability as compared to CT and MRI {Sargent 1997, Bakker 1998, Bakker 1999}. Ultrasonography, however, is the test of choice for the diagnosis of ADPKD, which is dependent on cyst quantity that exceeds the age-dependent incidence in the general population {Ravine 1994}.

More reproducible measurements of TKV can be obtained with CT and have been used to follow up the progression of ADPKD {Sise 2000}. However, this modality exposes patients to ionizing radiation.

The exposure to ionizing radiation can be avoided through MRI. Through this modality, TKV is measured by tracing the kidneys in sequential images and counting voxels, the three-dimensional equivalent of pixels {King 2000}.

The accuracy and precision of ultrasonography in assessing TKV in ADPKD compared with MRI was determined as part of the CRISP studies {O'Neill 2005}. Ultrasonography and MRI were performed at baseline and one year on 230 subjects with ADPKD. Ellipsoid-based measures of TKV were calculated utilizing US length, width, and depth, and sequential transverse images were used to measure TKV and TCV directly. These were compared with MRI measurements of TKV and TCV. Variability between different sonographers reading the same images ranged from 18-42%. Correlations between US and MRI volumes were 0.88 and 0.89. For the ellipsoid method, US TKV was 11% greater than MRI TKV, with a SD of 34%. For the direct method, mean difference was 9%, with a SD of 27%. None of the correlations with MRI TKV improved when subsets based on kidney size were analyzed. Sonographic measurements taken at baseline and at one year showed a mean increase in TKV during one year, but these were greater than the increase in TKV measured by MRI. Each measurement (ie length, depth and width) used to calculate the ellipsoid TKV also increased after one year. Variability in TKV change was much greater for US than MRI, again reflecting the poorer reproducibility of US. However, the variability in change in length was much less than that in width or depth. It was concluded that US measurement of TKV in patients with ADPKD is less accurate than MRI and lacks the precision necessary to measure short-term disease progression. However, US can provide an estimate of TKV that reflects severity and prognosis in individual patients.

To determine the reliability and accuracy of MRI measurements in the CRISP study, standardization studies were conducted in phantoms and four subjects who traveled to each participating clinical center.

Both large and small phantoms were measured by two analysts. For the small phantoms, each of four phantoms was imaged twice and measured twice by each of two analysts (4 _ 2 _ 2 _ 2 _ 32 measurements); the same design was used for the large phantoms. For measuring the small phantom balloon cysts, each of four phantoms was imaged twice and measured once by each of the two analysts (4 _ 2 _ 2 _ 16 measurements); the same design was used for the large phantoms. Three analysts evaluated the images from the standardization subjects for kidney and cyst volume. A two-thirds fractional design was used to examine the images, as every image was analyzed by two of the three analysts and each combination of analysts examined one third of the images. Phantom studies demonstrated high accuracy in measurement of total renal and cyst volumes by MRI imaging. The two sets of images (obtained from different positioning of the two types of phantoms) were measured two different times by two different analysts using the stereology technique to determine total renal volume. The collection of 64 volume measurements was examined using variance components methods in SAS PROC MIXED. Using this approach, the reliability coefficient for "analyst" was 0.994. The reliability coefficient for "positioning" was 0.992. For the balloon (a model for the renal cysts) components of the phantoms, balloons in the two sets of images were measured once by the two analysts so that 32 measurements were available for analysis. With these data, the reliability coefficient for the "analyst" component was 0.892. The variance component for "positioning" was too small for a valid estimate and no reliability value was computed.

The mean age of the four participating subjects (three women, one man, three Caucasians, one African American) was 36 ± 8.4 years; range, 21 to 45 years. Excellent reliability of measurement was found for all clinical variables. The reliability of the measurement process was examined using the variance

components method. For the TKV measurement using stereology, the reliability for "analyst" was 0.998. For the TCV measurement, the reliability for "analyst" was 0.961 and "location" was 0.962 {Chapman 2003}.

TKV measurements for CRISP were provided using a high level of standardization based on image analysis techniques established by Dr. Bae, the director of Image Analysis for both the CRISP and HALT PKD studies.{Grantham 2006a,b}. Strict process control measures for definition of acceptable imaging acquisition for TKV determination were developed under the leadership of Dr. Bae.

Figure 23 and Figure 24 provide a high-level visual perspective of the mix of imaging modalities for all subjects (one or more images), and for the subjects with two or more images.

Figure 23: All Subjects (1 or more images): Time Span and Modalities of Images (n=2355)
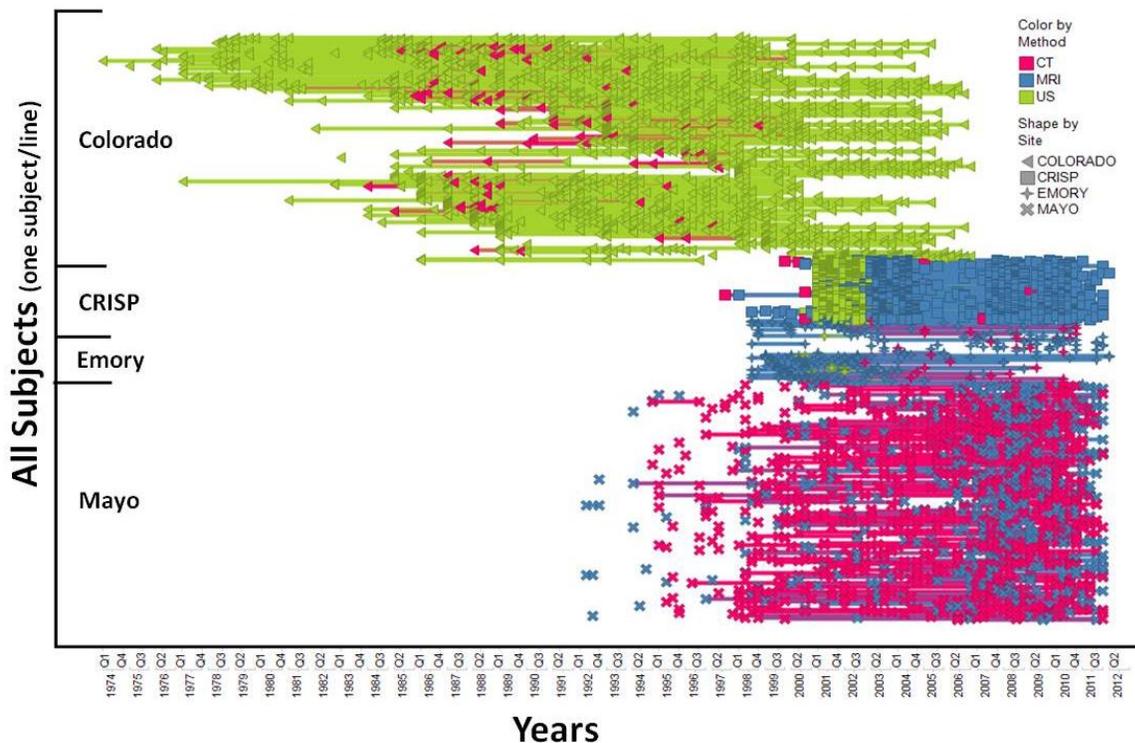
Figure 24: Subjects with Two or More Images: Time Span and Modalities of Images (n=1182)
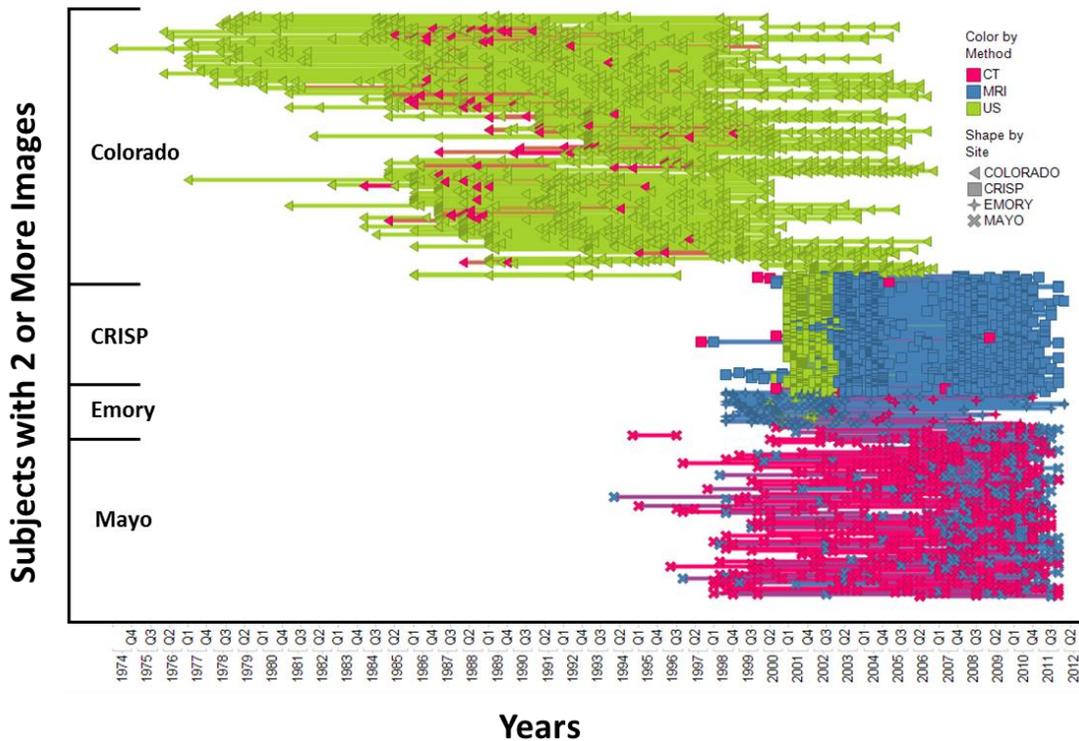
There were 2355 subjects with one or more images. Table 13 provides a summary of the number of images per subject, and Table 14 indicates the number of unique subjects, grouped by the number of images available, and the duration (in time) between their first and last image.

Table 13: Number of Images per Subject

Number of Images*	Number of Unique Subjects (n=2355)
1	1127
2	647
3	172
4	94
5	64
6	141
7	46
8	32
9	23
10	3
11	3
12	3

*Note: Images taken on the same day are counted as one image

Table 14: Number of Subjects by Number of Images and Time between First and Last Image

Number of Images*	Number of Unique Subjects (Time Between First and Last Image)					
	Subjects ≤ 6 months	Subjects > 6 months and ≤ 3 years	Subjects > 3 years and ≤ 5 years	Subjects > 5 years and ≤ 9 years	Subjects > 9 years	Total
2	46	230	156	163	52	647
3	0	33	17	58	64	172
4	0	16	21	22	35	94
5	0	0	7	35	22	64
6	0	0	12	79	50	141
7	0	0	0	21	25	46
8	0	0	0	6	26	32
9	0	0	0	2	21	23
10	0	0	0	0	3	3
11	0	0	0	0	3	3
12	0	0	0	0	3	3
Total	46	279	213	386	304	1228

*Images taken on the same day are counted as one image

In summary, studies provided in this submission document describe the close correlation and high level of agreement between simultaneous MR and CT measures in 43 adult ADPKD individuals (p. 89, Irazabal *et al.,* Journal of American Society of Nephrology, 2012) and between simultaneous MR and US measures in 230 CRISP participants (p. 84, O'Neill *et al.,* 2005). Both studies showed excellent agreement (MR vs. CT, < 3.7% difference between measures), but with increased variability in the US measurements. Differences found between modalities were significantly less (almost 100 fold) than the interval magnitude of TKV found to associate with future reduction of GFR. These data indicate that modality choice for TKV measures should not impact the predictability of TKV for renal progression in ADPKD.

O'Neill *et al*. also previously reported that US measurements of TKV in patients with ADPKD lacked the precision necessary to measure short-term disease progression; nonetheless, in this evaluation TKV measured by US was highly correlated with TKV measured by MRI (r = 0.88 – 0.89) {O'Neill 2005}.

Further, to understand potential differences in predictive accuracy from using TKV data collected by different imaging modalities, separate Kaplan-Meier plots, multivariate Cox models, and ROC analyses were done for TKV measured by US and compared with analyses based on TKV measured by MRI or CT. These analyses were done for each of the three endpoints and are shown in sections 5.1.4, 5.2.4, and 5.3.4. No difference was found in the predictive accuracy of models based on TKV measured by U/S

compared to TKV measured by MRI or CT. Based on the results of these analyses, and the previous modeling done in Appendix 8.5, subsequent modeling was performed using TKV data from all three imaging modalities.

These results suggest that baseline TKV (regardless of modality), as well as baseline age and baseline eGFR, are able to accurately predict the risk of 30% and 57% worsening of eGFR, as well as ESRD over a prolonged follow-up period.

4.3.1   Image Modality Settings

CRISP I and II: Please refer to the summaries of CRISP I and CRISP II protocols in Section 4.1.4.

Patient Registries:

At Emory University and Mayo Clinic, TKV measurements for registry subjects were obtained using point counting stereology from T1-weighted or CT images as described by the CRISP study group {Chapman 2003}. This point counting methodology is applicable to images obtained both by MRI and CT with or without contrast. Slight differences in TKV (~5% larger after gadolinium) are noted between studies with and without contrast; the difference was largest in kidneys of less than 750 cc {Bae 2009}. Smaller differences are anticipated for CT scans with and without iodinated contrast because the increase in signal due to iodinated contrast is less than that for gadolinium in MRI. At University of Colorado, TKV measurements were obtained using ultrasound and calculated using a standard formula for a modified ellipsoid {Fick-Brosnahan 2002}. In addition to the calculated TKV, measurements such as length, medial-lateral width, and anterior-posterior width for each kidney are available for all methodologies in all centers. It is recognized that ultrasound determination of TKV is less precise than MRI; however, we will explore the utility of kidney length in the model as well as using ultrasound measurements of TKV in a semi-quantitative fashion {O'Neill 2005}.

4.3.2   Image Acquisition and Reconstruction Parameters

Table 15: Ultrasound

Dataset/Site	University of Colorado PKD Registry Data
Settings and Acquisition Parameters	1985-1990: Abdominal ultrasonography was performed utilizing a Picker Digital Imaging II ultrasound scanner with a multi-format camera and high frequency transducers of 7.5 -10 MHz. 1990- : Abdominal ultrasonography was performed utilizing Acuson 128 real time ultrasound equipment. Depending on the subject size and ability to obtain adequate penetration, sector scanners ranging from 2.5 – 5.0 MHz were used. The highest frequency transducer that permitted adequate penetration and evaluation of the kidney was employed.
Acquisition of Volume Measurements	The kidneys were examined in both longitudinal and transverse planes. If enlargement of the organs did not permit complete evaluation of size using sector transducers, then static images were obtained through the kidneys using similar transducers. Volume was determined from

	maximum length (L), width (W), and diameter (D), using the formula for a modified ellipsoid (4/3π x (anteroposterior diameter/4 + width/4)2 x length/2. Length and width were obtained from longitudinal images whereas depth was obtained from transverse images of the mid kidney acquired in the plane perpendicular to the longitudinal plane. Kidney volume was also determined directly by measuring the cross-sectional area of the kidney in sequential transverse images and multiplying the sum by the slice interval of 1 cm. TKV was calculated from the sum of volume for the left and right kidneys. All measurements were made by the radiologist Dr. Manco-Johnson during scanning and all volumes calculated also by Dr. Manco-Johnson.
Data Storage	Access Database
Methods to validate measurements	TKV measurements were acquired using the above standardized protocol developed by the radiologist Dr. Manco-Johnson. All measurements and TKV calculation were performed by Dr. Manco-Johnson, ensuring consistency of methodology across longitudinal studies and eliminating inter-reader variability.
Assessment of variability	All measurements were obtained by a single reader.

Table 16: CT

Dataset	Mayo Clinic
Acquisition of Volume Measurements	CT images were retrieved to a work station and thoroughly inspected to determine if image quality was adequate for analysis (i.e., incomplete coverage). TKV was determined from 5-10 mm axial images with the stereology technique using Analyze software. All TKV determinations were made by Dr. M. Irazabal or image analyst A. Harmon.
Methods to validate measurements	To validate TKV measured on CT, we compared TKV determined on CT and MRI (validated with CRISP) in 43 Mayo patients in which a CT and MRI image was available within 15 days from each other. Difference in TKV (MRI-CT) was within 3.1% below to 3.6% above true TKV (MRI, CT mean). Combined percent measurement error = 1.53 {Irazabal 2012).
Assessment of variability	
Intra-Patient	Assessed with intra- and inter-observer variability.
Intra-reader	For assessment of intra-observer variability, repeated measurements at least 30 days apart were performed by the same observer. For case selection, TKVs were stratified into three groups based on their kidney size (combined right and left kidney volumes ≤750, 750 to 1500, >1500 ml). Repeated cases were selected randomly as follows: 3.5% patients from the small kidney-size group, 3% from the medium kidney-size group, and 3.5% from the large kidney-size group for a total of 10% of cases. Average intra-observer variability for CT

	(measurement error) = 0.97%.
Inter-reader	For inter-observer variability, repeated measurements were performed by two different observers following the same criteria as for intra-observer variability. Average inter-observer variability for CT (measurement error) = 1.57%.

Table 17: MRI with Gadolinium

Dataset	Mayo Clinic
Acquisition of Volume Measurements	MR images were retrieved to a work station and thoroughly inspected to determine if image quality was adequate for analysis (presence of artifacts, respiratory motion, and incomplete coverage). When T1-weighted post-gadolinium images were available and acceptable, TKV was determined from 3 mm coronal images with the stereology technique using Analyze software. All TKV determinations were made by Dr. M. Irazabal or image analyst A. Harmon.
Methods to validate measurements	In 27 Mayo Clinic patients from CRISP, TKV measurements were performed on CRISP baseline images following CRISP image analysis protocol (previously validated for reliability and accuracy) and results were compared against each other. Inter-observer/Center variability – measurement error (Mayo-CRISP) was calculated as follows: $$\frac{\lvert \Delta TKV \rvert}{\text{Ave. TKV}}$$ (0.91 %)
Assessment of variability	
Intra-Patient	Assessed with intra- and inter-observer variability.
Intra-reader	For assessment of intra-observer variability, repeated measurements at least 30 days apart were performed by the same observer. For case selection, TKV were stratified into three groups based on their kidney size (combined right and left kidney volumes ≤750, 750 to 1500, >1500 ml). Repeated cases were selected randomly as follows: 3.5% patients from the small kidney-size group, 3% from the medium kidney-size group, and 3.5% from the large kidney-size group for a total of 10% of cases. Average intra-observer variability for MRI with gadolinium (measurement error) = 0.88%.
Inter-reader	For inter-observer variability, repeated measurements were performed by two different observers following the same criteria as for intra-observer variability. Average measurement error for MRI with gadolinium = 0.95 %.
Dataset	Emory University
Acquisition of Volume Measurements	MR images were retrieved to a work station and thoroughly inspected to determine if image quality was adequate for analysis (presence of artifacts, respiratory motion, and incomplete coverage). When T1-

	weighted post-gadolinium images were available and acceptable, TKV was determined from 3 mm coronal images with the stereology technique using Analyze software. All TKV determinations were made by Dr. Kristhla Arya or image analyst A. Mittal.
Methods to validate measurements	In 30 Emory patients from CRISP, TKV measurements were performed on CRISP baseline images following CRISP image analysis protocol (previously validated for reliability and accuracy) and results were compared against each other. Inter-observer/Center variability – measurement error (Emory-CRISP) was calculated as follows: $$\frac{\lvert\Delta TKV\rvert}{Ave.\ TKV}$$ (0.97 %)
Assessment of variability	
Intra-Patient	Assessed with intra and inter-observer variability.
Intra-reader	For assessment of intra-observer variability, repeated measurements at least two weeks apart were performed by the same observer. For case selection, TKV were stratified into two groups based on their kidney size (combined right and left kidney volumes ≤1500 and >1500 ml). Repeated cases were selected randomly as follows: 10 patients from the small kidney-size group, and 10 from the large kidney-size group Average intra-observer variability for MRI with gadolinium (measurement error) = 0.97%.
Inter-reader	For inter-observer variability, repeated measurements were performed by two different observers following the same criteria as for intra-observer variability. Average measurement error for MRI with gadolinium = 0.99 %.

Table 18: MRI without Gadolinium

Dataset	Mayo Clinic
Acquisition of Volume Measurements	MR images were retrieved to a work station and thoroughly inspected to determine if image quality is adequate for analysis (presence of artifacts, respiratory motion, and incomplete coverage). TKV was determined from 3 mm non-contrast enhanced coronal T1-weighted images when adequate, with the next choice being 3 mm coronal T2-weighted images with the stereology technique using Analyze software. All TKV determinations were made by Dr. M. Irazabal or image analyst A. Harmon.
Methods to validate measurements	
Assessment of variability	
Intra-Patient	Assessed with intra and inter-observer variability.
Intra-reader	For assessment of intra-observer variability, repeated measurements at

	least 30 days apart were performed by the same observer. For case selection, TKV were stratified into three groups based on their kidney size (combined right and left kidney volumes ≤750, 750 to 1500, >1500 ml). Repeated cases were selected randomly as follows: 3.5% patients from the small kidney-size group, 3% from the medium kidney-size group, and 3.5% from the large kidney-size group for a total of 10% of cases. Average intra-observer variability for MRI without gadolinium (measurement error) = 0.98%.
Inter-reader	For inter-observer variability, repeated measurements were performed by two different observers following the same criteria as for intra-observer variability. Average inter-observer variability for MRI without gadolinium (measurement error) = 1.32%.

4.4   Data Analysis Methodology

The steps and methods that were used for the analysis qualification of TKV are presented in this section. The following data rules were used to construct datasets.

1. **Baseline Age**: age for the first TKV measurement for a subject within a population of interest.

2. **Baseline TKV**: first TKV measurement for a subject within a population of interest.

3. **Baseline eGFR**: eGFR was estimated using the CKD-EPI equation (see Appendix 8.1) from the first valid serum creatinine measurement on or after, and within 365 days of the baseline TKV.

4. **Computation of 'date of last follow-up' for analysis endpoints:**

   - **30% worsening of eGFR:** eGFR values were derived using serum creatinine. This endpoint represents a 30% decline in eGFR relative to the baseline. A subsequent measurement within any timeframe was required to confirm that the original decline was not just transient.

   - **57% worsening of eGFR**: eGFR values were derived using serum creatinine. This endpoint represents a 57% decline in eGFR relative to the baseline. A subsequent measurement within any timeframe was required to confirm that the original decline was not just transient.

   - **ESRD:** if ESRD date was not specifically provided, the patient was considered not to have reached ESRD as of the last 'interaction' date was identified for the subject by searching the related CDISC domains, as well as the extra follow-up data files provided by the sites.

5. **Endpoint Verification**: for the above endpoints of interest, 'date of last follow-up' could not be later than the Death Date (when provided).

6. **Endpoint measurements before the Baseline TKV**: since the goal was to correlate endpoints with TKV, only endpoint measurements that occurred after the Baseline TKV measurement were considered for the analysis. Events that occurred before the Baseline TKV were still summarized for completeness, but were not modeled.

7. **Height Measurements**: For adults (age 18 or over), any available height measurement was acceptable for use in evaluation of height-adjusted TKV. For calculation of eGFR in pediatric subjects, please see #8.

8. **Calculation of Pediatric eGFR – Merging Data**: serum creatinine and height measurements were not always available on the same date, but both values were required for the pediatric eGFR calculation. First, a check was made to determine whether there were multiple sCR values on the same date. Values were averaged if there were multiple measurements. Then, the height measurement nearest to the time of the sCR measurement was used to calculate eGFR and CKD. If height measurements were recorded on exactly the same number of days before and after a given sCR measurement, then height values were averaged, and the mean result was used to calculate eGFR and CKD for that sCR value. The maximum difference allowed was one year.

9. **Merging Covariate Data (that may vary over time) with Baseline TKV Data**: Lab and clinical measurements required for TKV correlation were not always available on the same date. The lab or clinical measurements that were nearest (before or after) to the image date were used. The maximum difference allowed was one year. If there were covariate data obtained at the same number of days before and after the image date, and/or there were multiple observations on one date, the observations were averaged.

10. **Lab or Vital Sign Measurements**: if there were multiple measurements on the same day that were different by more than 10%, the data were reconciled with the individual site PI. Otherwise, values were averaged. This rule did not apply to BP measurements (see rule 4e).

11. **Determining Dates for Partial Date Fields:** if the month was missing, it was assigned as the first month of the year (January). If the day was missing, it was assigned as the first day of the month. For example, a date provided as '2000' was assigned as '2000-01-01'. (Note: If there were cases where this may cause a conflict with the Death Date, or the elimination of a priority event, the C-Path Data Management group was contacted. For example, if a subject has a Death Date of '2000,' and a full ESRD date of '2000-04-18,' the data were resolved with the individual site PI.)

12. **Modality Population Definitions:**

    a. **All Modalities:** all subjects from the PKD database who have a least one image measurement (regardless of modality).

    b. **CT-MRI Modalities**: Subjects with at least one CT (computer assisted tomography) or MRI image. CT and MRI were treated as equivalent.

    c. **US Modality**: Subjects with at least one ultrasound (US) image.

13. **Missing Data / Sensitivity Analysis Notes:**

    a. For the 30% and 57% decline in eGFR, only confirmed endpoints were utilized. A 30% decline in eGFR relative to baseline was used to derive a binary endpoint.

    b. All sites utilized USRDS and the National Death Index to obtain information on subjects lost to follow-up.

c. A subsequent measurement was required to confirm the original 30% decline in eGFR (referred as the "restrictive" definition of the endpoint). A sensitivity analysis was performed based on a dataset in which a 30% worsening of eGFR was defined as a 30% decline in eGFR relative to the baseline without the need of a subsequent confirmatory measurement (referred as the "non-restrictive endpoint"). This sensitivity analysis was performed to examine potential differences in outcome between the "restrictive" and "non-restrictive" definition of a 30% worsening of eGFR endpoints. A similar sensitivity analysis was performed for the 57% worsening of eGFR. Additional details on the sensitivity analysis of the 30% and 57% worsening of eGFR are presented in sections 5.1.3 and 5.2.3, respectively.

4.5   Data Sets and Exploratory Data Analyses

4.6   PKDOC-CDISC Database and Datasets

The PKDOC, in collaboration with the Clinical Data Interchange Standards Consortium (CDISC), has aggregated data from multiple clinical trials and clinical registries into a common database in a standard CDISC Study Data Tabulation Model (SDTM) (www.cdisc.org).

The current database was constructed according to SDTM standards for the data elements needed for ADPKD. Data was aggregated from the CRISP1 and CRISP2 studies as well as multiple, longitudinal, well-characterized research registries maintained over decades by the leading institutions conducting clinical investigation in ADPKD (University of Colorado - Denver, Emory University, and Mayo Clinic). Refer to Appendix 8.2 for a summary of components of the database.

Each contributing organization's data were mapped into CDISC format using available translation tools to aid in this process. Once translated, the data were electronically uploaded via a secure connection to the C-Path online data repository. All data submitted were validated by a Quality Control process to ensure its integrity and quality before being added to the database. The data are stored at DataPipe, Inc., an industry-leading national data hosting company (http://www.datapipe.com). The database is protected with secure, industry standard firewalls, protocol encryption, anti-service attack mechanisms, and forward and backward proxies.

There is inherent risk in mapping data to a new format. To mitigate this risk, PKDOC worked closely with the sites to make sure they understood the source-to-target logic. This included referring to the original CRF to ensure data collection context was considered on an item by item basis. Regularly scheduled group calls including all sites were held in order to agree on a unified approach and to make sure that disparate data were not being "shoehorned" into the standard in a way that changed clinical meaning. Whenever the poolability of the legacy data was questioned, the data standardization group consulted with the clinical PIs to ensure agreement on a strategy for pooling.

Data elements from multiple case report forms were consolidated into standard data elements through a consensus process. The FDA and NIH have been active supporters and participants in this process. The consensus CDISC data standards were released for global comment on October 22, 2012 and finalized on November 19, 2013. A technical review was performed on January 28, 2013. Version 1.0 of the Polycystic Kidney Disease Therapeutic User Guide was released on April 17, 2013.

In many respects, these extensive efforts to standardize and integrate the observational data utilized concepts and criteria similar to the "STrengthening the Reporting of OBservational studies in Epidemiology" (STROBE) guidelines. Based on a recommendation from the EMA, the PKDOC did a comparison with STROBE and believes that the key STROBE criteria were achieved (**Table 19**).

Table 19: Comparison of PKDOC Methods with STROBE Methodologies

Area	#	STROBE Recommendation	PKDOC Comments / Ref
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Designated as registry, not specifically stated as COHORT
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Pages 12-19 and section 4.1
Introduction			
Background	2	Explain the scientific background and rationale for the investigation being reported	Pages 12-19 and 38
Objectives	3	State specific objectives, including any pre-specified hypotheses	Pages 12-19, and 38
Methods			
Study design	4	Present key elements of study design early in the paper	Pages 12-19, 38-40, and 71-106
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Section 4, beginning page 70
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Section 4, beginning page 70
		(b) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pages 102-104, 108
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Section 4, beginning page 70; imaging page 93
Bias	9	Describe any efforts to address potential sources of bias	We believe the registries are representative of all PKD patients. There were no

			exclusion criteria. See page 17.
Study size	**10**	Explain how the study size was arrived at	All available subjects with measurement of TKV, page 70
Quantitative variables	**11**	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 102
Statistical methods	**12**	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, explain how loss to follow-up was addressed (e) Describe any sensitivity analyses	Page 102 and 108 Page 83 Item 12a (p 103) Item 12b (p103) Item 12c (p103)
Participants	**13**	(a) Report numbers of individuals at each stage of study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	Page 18 Included in written response to EMA
Descriptive data	**14**	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (e.g., average and total amount)	Page 85 Page 110 Page 110 (Section 5)
Outcome data	**15**	Report numbers of outcome events or summary measures over time	Page 110 (Section 5)
Main results	**16**	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included. (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 110 (Section 5)

Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Joint Modeling, Page 110 (Section 5)
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 151 (Section 6)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	Page 151 (Section 6)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 151 (Section 6)
Generalizability	21	Discuss the generalizability (external validity) of the study results	Page 151 (Section 6)
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 14

4.6.1   Descriptive Statistics: Baseline characteristics

Demographic data from the CRISP study and patient registries from University sites (University of Colorado - Denver, Emory University, and Mayo Clinic) were merged. ADPKD patients who both enrolled in the CRISP study and provided demographic information as part of patient registries in university sites (Emory University and Mayo Clinic) were identified as 'common subjects.' These data were handled carefully to avoid duplication of records. Patients from the registries at Emory University and Mayo Clinic who subsequently participated in CRISP required adjudication of events recorded in the registries that were also later recorded in CRISP. In general, the dates and details of Clinical Events recorded in real time in the registries were prioritized over that same event that was subsequently captured as Medical History in CRISP. The adjudication process for common subjects is discussed in greater detail in Table 12.

The following demographic data were summarized with descriptive statistics: age, sex, race, ADPKD mutations (*Pkd1*, *Pkd2*, or unknown), and eGFR (see Section 4.2).

eGFR was derived using the original 4-variable MDRD equation for creatinine methods that are not calibrated to an IDMS reference method. For creatinine methods calibrated to an IDMS reference method, the IDMS-traceable MDRD study equation was used to derive eGFR. (See National Kidney Disease Education Program, NKDEP eGFR Calculators).

The dates for introduction of IDMS-traceable creatinine methods for Emory University and Mayo Clinic are April 8, 2009 and October 18, 2006, respectively. All creatinine measurements from the University of Colorado were done prior to the introduction of IDMS traceability in 2005. CRISP creatinine measurements were done at each of the four institutions involved: Emory University, Mayo Clinic,

University of Alabama, and Kansas University. The dates for introduction of IDMS- traceable creatinine methods for Emory University and Mayo Clinic are as above. The date for introduction of IDMS-traceable creatinine methods for University of Alabama and Kansas University are April 15, 2008 and March 11, 2008, respectively.

4.6.2   Descriptive Statistics: ADPKD Disease Outcome

Baseline demographics are summarized with the following descriptive statistics: number of observations (n), mean, medium, and standard deviation (SD). Categorical data are summarized with the following descriptive statistics: number of observations (n), and percentage (%). Descriptive statistics are provided for each study/site and overall (CRISP and patient registries combined).

Longitudinal measures of kidney function (eGFR and TKV) are presented using scatterplots (linear and semi-log scale). A locally weighted scatterplot smoothing (LOESS) curve is provided to identify potential trends over time.

ADPKD disease outcomes are summarized with descriptive statistics, and are provided for the following disease outcomes of ADPKD.

- 30% Worsening of eGFR

- 57% Worsening of eGFR

- End-Stage Renal Disease

Note: Time to 30% and 57% worsening of eGFR were derived based on individual eGFR data provided in the database or calculated as described in Section 4.3.

If a disease outcome was repeated in a patient, the first onset was used for descriptive statistics.

4.7   TKV-Disease Model and Validation

4.7.1   Cox Models

Cox models were developed for patients with at least one TKV measurement.

Univariate Cox models (1-by-1) were developed in a first step to assess the effect of various candidate predictors for the probability of disease outcome. The following predictors were considered: baseline TKV (ln-transformed and untransformed), height-adjusted baseline TKV (ln-transformed and untransformed), baseline age (age at first TKV measurement), baseline eGFR (eGFR at first TKV measurement), sex, race (white and non-white), and genotype (PKD1 and PKD2). The predictive performance of individual terms was assessed by deriving receiver-operating characteristics (ROC) at one and five years.

In addition, multivariate Cox models were constructed by including relevant predictors in the model to tease out potential confounding effects and for testing potential interaction terms between baseline TKV, baseline age and baseline eGFR. Models with different interaction terms were compared by deriving Akaike Information Criterion (AIC) and ROC values at one and five years. The final interaction model was selected based on the AIC and ROC values. Hazard ratios for individual predictors were derived with the final multivariate Cox model with interactions.

Joint modeling and cross-validation were performed using R$^{®}$ 3.0.2 (64-bit).

4.7.2   Joint Modeling of Longitudinal TKV and Probability of Disease Outcome

Joint Modeling

Joint modeling is considered the gold standard method for assessing the effect of longitudinal time-varying covariates (e.g., TKV) in a time-to-event analysis of clinical endpoint (Sweeting *et al*., 2011; Tsiatis, & Davidian, 2004).

Patients with at least two TKV measurements separated by at least six months were included in the analysis.

The following models were developed:

> **TKV Model**
> - A linear mixed-effect model with a random intercept (baseline ln-transformed TKV) was used to fit ln-transformed TKV values over time.

> **Event Model**
> - Association parameter between predicted TKV at the time of event was modeled using various hazard functions such as Weibull and piecewise linear.
> - Baseline age, baseline eGFR and interaction terms were tested in the model.

A p-value of 0.05 was used for statistical inferences. Standard likelihood ratio tests and the AIC were used for model discrimination when appropriate. Missing data were not imputed.

Cross-Validation Methodology

In a first step, data splitting was performed to allow cross-validation of predictions made with the model. Cross-validation was performed using a five-fold or ten-fold cross-validation approach {Breiman *et al.,* 1992}.

The following steps were performed:

1. Data was split into five or ten parts with roughly equal number of subjects. Splitting was stratified to maintain a similar proportion of patients from the CRISP and registry datasets in the reference and test datasets. Each fold served as a test dataset in the following steps, while the rest of the data consisted of the training dataset (i.e., the four or nine other folds).

2. The joint model (including relevant prognostic factors identified based on the multivariate Cox model) was fitted to the training dataset (4/5 or 9/10 of the folds).

3. Prediction of disease outcomes for the test dataset (5th or 10th fold, not used in the fit) was performed by simulating from the joint model using each individual prognostic factors (longitudinal TKV data, baseline age and baseline eGFR) from the test dataset.

   a. Model-based predicted probabilities in the test dataset were compared to observed disease outcomes in the test dataset. Predictive performance of the joint model was assessed by computing descriptive statistics of observed vs. predicted probability of

disease outcomes (precision and accuracy). Mean prediction errors (MPE) are computed as: (pred_val – obs_val)/obs_val * 100%, where obs_val and pred_val are the observed and predicted percentiles at the desired quantile (time) over the Test (or validation) group in the fold. Root mean square errors (RMSE) are computed as:

$$RMSE(\%) = \left[N^{-1} \cdot \sum_{i=1}^{N} (pe_i)^2 \right]^{\frac{1}{2}}$$

b. The above steps were repeated for each fold.

Joint modeling and cross-validation was performed using the JM package in R$^{®}$ 3.0.2 (64-bit).

4.7.3 Quality Control and Archiving

Quality control on final derived datasets and final analysis scripts was performed according to Pharsight's SOP-053 (Quality Control and Quality Assurance Inspection) using a double programming approach.

5   Results – Modeling and Analysis

The results of the statistical analysis and modeling are included in this section. Analyses were done for three different outcome measures (30% reduction in eGFR, 57% reduction in eGFR, and ESRD) using all three imaging modalities (US, CT and MRI). Based on the request from the regulatory agencies to verify equivalence of the imaging modalities, each endpoint dataset (30% reduction in eGFR, 57% reduction in eGFR, and ESRD) was divided into two datasets (MRI/CT and US) and a Cox analysis was performed. It is important to emphasize that the two imaging modality data subsets do not reflect a comparison of the same subjects using different modalities, but in fact are different subject populations. In addition, critical characteristics of these two subsets differ with regard to age and kidney function, where the MRI/CT subgroup is older and has lower kidney function or more progressive renal insufficiency. By definition the MRI/CT and US modality datasets are much smaller in sample size as compared to the combined modality dataset. In the subset of US and MRI/CT, the number of interaction terms relevant in the overall combined model was too numerous than could be estimated from the data and resulted in model "over-parameterization" (i.e., over-fitting of the data). Simpler models were then tested to avoid "over-parameterization". Where applicable, this is noted in the text of the endpoint sections.

The three outcome measures tested are:

- 30% worsening of eGFR

- 57% worsening of eGFR (doubling of serum creatinine)

- End-Stage Renal disease (start of dialysis or kidney transplant)

5.1   30% Worsening of eGFR

5.1.1   30% Worsening of eGFR - Endpoint Definition and Exploratory Analyses

Based on the combined modality dataset, a 30% decline in eGFR relative to baseline was used to derive a binary endpoint. A subsequent measurement was required to confirm the original 30% decline in eGFR (referred as the "restrictive" definition of the endpoint). TKV values measured by MRI, CT, or US modalities were used in the analysis.

A total of 2355 patients with at least one TKV measurement (all modalities) in the database were available. A total of 1215 patients with missing covariates were excluded, of which 664 had missing baseline eGFR. Overall, the analysis dataset included 1140 patients of which 361 (31.7%) patients had a 30% worsening of eGFR. There were no missing covariates of interest in the final dataset, but there were two patients with missing height and 466 patients with missing genotype information.

After the creation of the 30% worsening of eGFR analysis dataset, the following baseline characteristics of the included patients were generated and are provided in Figure 25.

Figure 25: Baseline Characteristics of Patients included in the 30% Worsening of eGFR Analysis
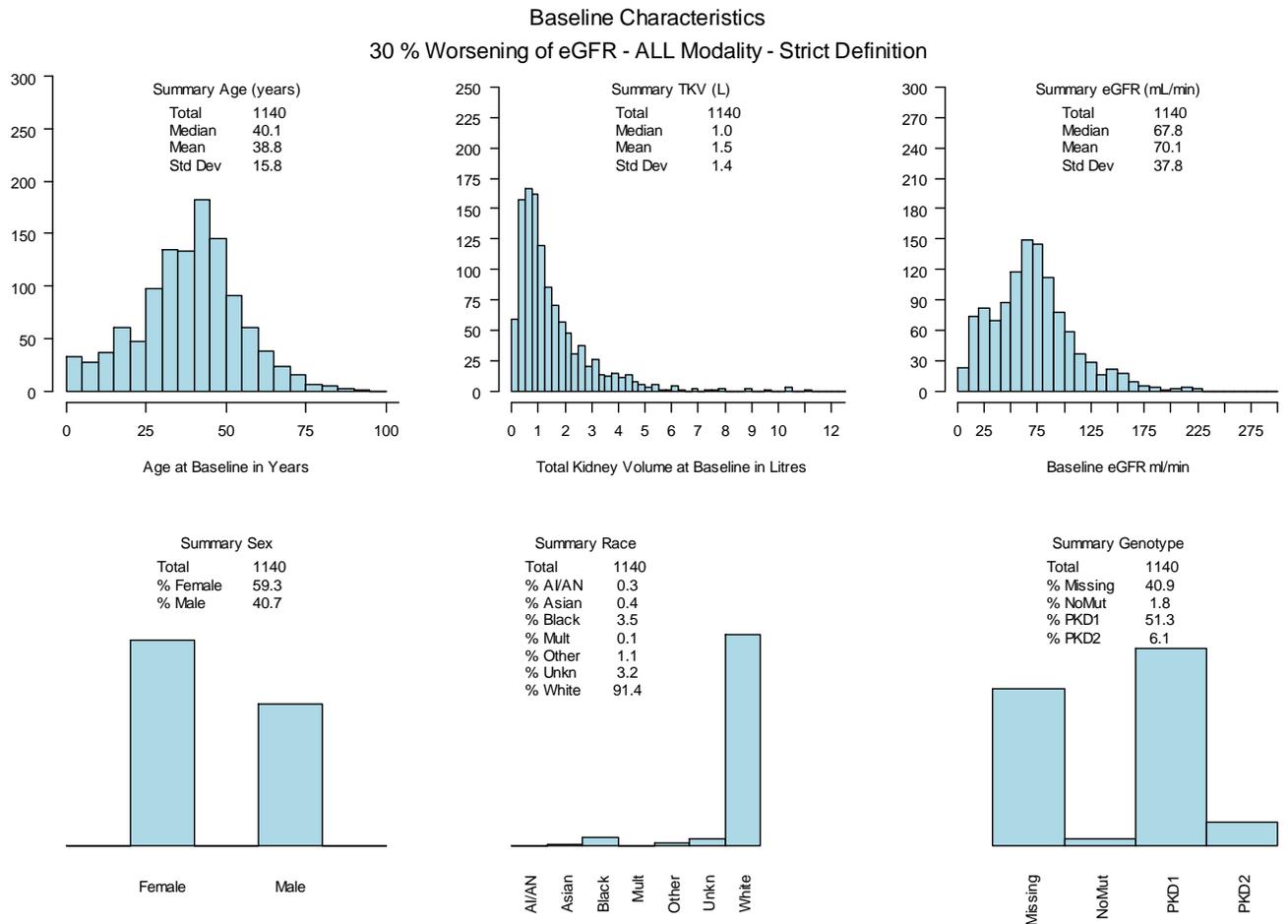
A Kaplan-Meier figure for the probability of avoiding a 30% worsening of eGFR as a function of years of follow-up is presented in **Figure 26**.

Figure 26: Kaplan-Meier Plot for the Probability of No Worsening of 30% eGFR as a Function of Years of Follow-Up
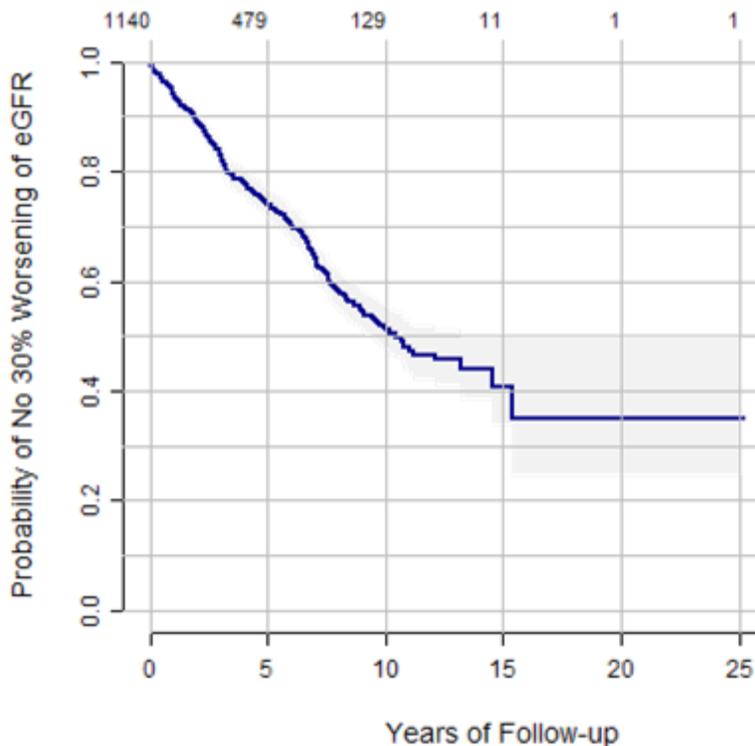
Years of follow-up were calculated relative to the first TKV measurement. A steep decrease in the probability of no 30% worsening of eGFR was observed within five years of follow-up. The probability of reaching 30% worsening of eGFR at five years of follow-up was approximately 25%.

A Kaplan-Meier figure for the probability of avoiding a 30% worsening of eGFR as a function of baseline TKV (< 1 or ≥1 L) and baseline eGFR (< 50 or ≥ 50 mL/min/1.73m^2) is presented in **Figure 27**.

Figure 27: Kaplan-Meier Plot for the Probability of No Worsening of 30% eGFR as a Function of Baseline TKV and Baseline eGFR

For patients with "preserved" kidney function (i.e., eGFR ≥ 50 mL/min/1.73m^2), the risk of a 30% worsening in ADPKD patients with larger TKV (≥ 1 L) was greater than that observed in patients with smaller TKV (< 1 L) (grey dashed vs. grey solid lines).

For patients with "reduced" kidney function (i.e., eGFR < 50 mL/min/1.73m^2), the risk of a 30% worsening of eGFR in ADPKD patients with larger TKV (≥ 1 L) was greater than that observed in patients with smaller TKV (< 1 L) (black dashed vs. black solid lines).

The above results suggest that TKV is prognostic for selecting patients most likely to progress to a 30% worsening of eGFR in populations with "preserved" (those mostly likely to be enrolled in a clinical trial) and "reduced" kidney function. Furthermore, the above results suggest that trial enrichment based on the selection of patient characteristics may potentially be applied to predict faster disease progression in sub-populations of interest.

A Kaplan-Meier figure for the probability of no 30% worsening of eGFR as a function of baseline TKV (< 1 or ≥1 L), baseline eGFR (< 50 or ≥ 50 mL/min/1.73m^2) and baseline age (< 40 or ≥ 40 years) is presented for information purposes in Appendix 8.6.

5.1.2   30% Worsening of eGFR - Univariate Cox Analysis

Univariate Cox models (1-by-1) were used in a first step to assess the effect of individual candidate predictors for the probability of a 30% worsening of eGFR ("restricted" definition of the endpoints). The following predictors were considered: baseline TKV (ln-transformed and untransformed), baseline height-adjusted TKV (ln-transformed and untransformed), baseline eGFR (eGFR at first TKV measurement), baseline age (age at first TKV measurement), sex, race (white and non-white), and genotype (no mutation reported, PKD1 and PKD2).

Results of the univariate Cox analysis are presented in Table 20.

Table 20: Univariate Cox Results for the Probability of a 30% Worsening of eGFR (All Modalities)

Covariate	N	P-Value	Sign	Hazard Ratio	Lower 95% CI	Upper 95% CI	ROC 1	ROC 5
Ln Baseline HA TKV	**1138**	<0.001	+	**2.52**	**2.18**	**2.92**	0.695	0.677
Ln Baseline TKV	**1140**	<0.001	+	**2.36**	**2.05**	**2.72**	0.689	0.675
Baseline HA TKV	1138	<0.001	+	1.07	1.06	1.08	0.659	0.589
Baseline TKV	1140	<0.001	+	1.44	1.37	1.52	0.655	0.590
Baseline eGFR	1140	<0.001	-	0.99	0.98	0.99	0.605	0.587
Baseline Age	1140	<0.001	+	1.02	1.01	1.03	0.588	0.585
Baseline Height	1138	<0.001	+	1.02	1.01	1.02	0.559	0.569
Sex	1140	<0.001	+	1.43	1.16	1.76	0.544	0.544
Genotype	674	0.142	-	0.68	1.46	2.21	0.523	0.520
Race	1140	0.571	+	1.13	0.73	1.74	0.504	0.503

Notes: HA= height-adjusted; N= sample size; ROC= are under receiver operator characteristic curves for predicting the outcome at year 1 or 5. Hazard ratio for Ln Baseline HA TKV and Ln Baseline TKV were derived for a difference of 1 log (i.e., 2.7-fold increase in HA TKV and TKV). Hazard ratio for Baseline HA TKV, Baseline TKV, Baseline eGFR, Baseline Age, and Baseline Height were derived for a 1 unit increment (i.e., mL/cm, L, mL/min, year, and cm, respectively). Hazard ratio for Sex (Male vs. female comparison), Genotype (PKD1 vs. no mutation), and Race (white vs. non-white) were derived.

The effect of ln-transformed baseline height-adjusted TKV and ln-transformed baseline TKV resulted in an improvement in model fit, as reflected in the higher ROC values as compared with the models based on the raw values. Because TKV growth appears to be exponential {Grantham 2006}, a log transformed change in TKV was a natural choice and it was shown to be more prognostic than a raw un-transformed TKV. Changes on a log scale reflect fold increases rather than absolute mls increase. For example, a change of 1 log would reflect a 2.718 fold increase (e.g., from 500 mls to 1359 mls or from 1000 mls to 2718 mls) and both would result in the same effect on the hazard ratio.

The effect of sex was statistically significant with a hazard ratio of 1.43 (suggesting a 43% higher probability of a 30% worsening in male patients).

All other covariates were statistically significant, with the exception of genotype (p=0.142) and race (p=0.571). Therefore, effects of genotype and race were not tested in the multivariate Cox model.

Results derived with the above univariate Cox models (1-by-1) should be interpreted with caution considering the confounding effects between covariates.

5.1.3   30% Worsening of eGFR - Multivariate Cox Analysis and Interaction Terms

In order to tease out confounding effects between baseline TKV and other covariates such as baseline age and baseline eGFR, a multivariate Cox analysis was performed for the probability of a 30% worsening of eGFR ("restricted" definition of the endpoints). Although ln-transformed baseline height-adjusted TKV and ln-transformed baseline TKV resulted in a similar predictive power (ROC values at one and five years) based on the univariate Cox analysis (refer to Table 21), ln-transformed baseline TKV was used in the multivariate Cox model since it was deemed more convenient to use in a clinical setting. As ln-transformed baseline TKV was our key predictor of interest, it was the first variable incorporated in the multivariate Cox model, and it was included in all subsequent models. Other covariates were tested through forward stepwise model building. Final parameters included in the multivariate Cox model and statistically significant interaction terms are presented in Table 21.

Table 21: Final Multivariate Cox Model Including Interaction Terms for the Probability of a 30% Worsening of eGFR

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	P-Value
Prognostic Factors					
Ln Baseline TKV	2. 755094	15.723	0.367392	7.5	6.40E-14
Baseline Age	0.26031	1.297	0.043426	5.99	2.00E-09
Baseline eGFR	0.096056	1.101	0.016639	5.77	7.80E-09
Interaction Terms					
TKV:Age	-0.02905	0.971	0.005861	-4.96	7.20E-07
eGFR:Age	-0.00079	0.999	0.00014	-5.65	1.60E-08
eGFR:TKV	-0.00994	0.990	0.002633	-3.77	0.00016

As shown in Table 21, the association of TKV with a 30% decline in eGFR is statistically significant, independent of age and eGFR. In addition to ln-transformed baseline TKV, the effect of baseline eGFR and baseline age were highly statistically significant (P<0.0001). It is well known that TKV, age, and eGFR are not completely independent. Therefore, the following interaction terms were tested in the multivariate Cox model: 1) interaction between ln-transformed baseline TKV and baseline age, 2) interaction between baseline eGFR and baseline age, and 3) interaction between baseline eGFR and ln-transformed baseline TKV. This interaction model resulted in the highest ROC1 and ROC5 values (0.7477 and 0.7006, respectively). A summary of the stepwise analysis, as well as different interaction models tested in the models are presented in Appendix 8.6.

Based on ROC values (predictive value of models) as well as Z and p-values (statistical contribution of individual covariates), these results indicate that ln-transformed baseline TKV is the most important prognostic biomarker of progression to 30% worsening of eGFR, though age and eGFR also contribute independent predictive information.

As shown previously, the effect of gender was statistically significant in the univariate Cox model, with males displaying a greater probability of a 30% worsening of eGFR than females. On the other hand, the effect of gender was not statistically significant (p=0.26677) in the multivariate Cox due to the well-known correlation between gender and TKV (i.e., men generally have larger kidneys than women).

Likewise, the effect of genotype on PKD is mediated by its effects on cyst number and growth. Therefore, genotyping (i.e., PKD1 vs. PKD2) adds little to imaging of TKV as a biomarker for predicting the natural history of the disease in individual cases as also reported for the CRISP population {Chapman 2012}.

Overall, the above results suggest that ln-transformed baseline TKV was the most important prognostic biomarker of disease progression in ADPKD patients. Baseline TKV, as well as baseline age and baseline eGFR can be used as inclusion criterion in clinical trials to identify patients likely to show a 30% worsening of eGFR during a clinical trial.

An exploratory full model analysis was performed to assess whether the prognostic value of ln-transformed baseline TKV was preserved after forcing other non-significant covariates into the final multivariate Cox model. After including the effect of sex (p=0.37) and race (p=0.89) in the multivariate Cox model, the p-value associated to ln-transformed baseline TKV remained highly significant (P<0.0001) (refer to Appendix 8.6).

Hazard ratios with 95% confidence intervals (CI) derived with the final multivariate Cox model are presented in Table 22.

Table 22: Hazard Ratios for the Probability of a 30% Worsening of eGFR

Parameters	Hazard Ratio	Lower 95% CI	Upper 95% CI
Prognostic Factors			
Ln Baseline TKV	15.723	7.652	32.303
Baseline Age	1.297	1.191	1.413
Baseline eGFR	1.101	1.066	1.137
Interaction Terms			
TKV:Age	0.971	0.960	0.983
eGFR:Age	0.999	0.999	0.999
eGFR:TKV	0.990	0.985	0.995

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively.

For each increase of 1 log TKV (i.e.TKV 1000 mls vs. 2738 mls), holding age and eGFR constant, there is an approximate 15-fold increase in the probability of a 30% worsening of eGFR. Baseline age and baseline eGFR were associated with hazard ratios of 1.297 and 1.101, respectively.

A sensitivity analysis was performed based on a dataset in which a 30% worsening of eGFR was defined as a 30% decline in eGFR relative to the baseline without the need of a subsequent confirmatory measurement (referred as the "non-restrictive endpoint"). This sensitivity analysis was performed to examine potential differences in outcome between the "restrictive" and "non-restrictive" definition of a 30% worsening of eGFR endpoints. Statistical outputs are provided in Appendix 8.6. Results of the exploratory analysis are summarized below.

- Overall, there were 2355 patients with at least one TKV measurement in the database. A total of 1170 patients with insufficient information were excluded, of which 664 had missing baseline eGFR. Overall the analysis dataset included 1185 patients with 514 (43.4%) 30% worsening of eGFR events. There were no missing covariate information except for two patients with missing heights and 484 with missing genotypes.

- Hazard ratios for the effect of ln-transformed baseline TKV, baseline age, and baseline eGFR derived with the "non-restrictive" definition of a 30% worsening of eGFR (12.813, 1.290 and 1.087, respectively) were consistent with those derived with the "restrictive" definition (15.723, 1.297 and 1.101, respectively).

5.1.4   30% Worsening of eGFR - Exploratory Analyses Based on MRI/CT and US Modalities

Based on the request from the regulatory agencies to verify equivalence of the imaging modalities, each endpoint dataset was divided into two separate datasets (MRI/CT and US) to perform a Cox analysis. It is important to emphasize that the two modality datasets do not reflect a comparison of the same subjects using different modalities, but in fact are primarily different subject populations. Critical characteristics of these two subsets differ with regard to age and kidney function, where the MRI/CT subgroup is older and has lower kidney function or more progressive renal insufficiency. In addition, the MRI/CT and US modality datasets are smaller in sample size relative to the "all modality" dataset (i.e., a combination of all modalities).

Exploratory analyses were performed based on a dataset including TKV values measured with MRI and CT vs. US modalities for a 30% worsening of eGFR endpoints ("restricted" definition). This exploratory analysis was performed to examine potential differences in outcome between the "MRI-CT" dataset relative to the "US" dataset. Similar analyses as described in Section 5.1.3 were performed. Summary results are presented in Appendix 8.6. A Kaplan-Meier figure for the probability of no 30% worsening of eGFR for the MRI/CT and US datasets are presented in **Figure 28**.

Figure 28: Kaplan-Meier Plot for the Probability of No Worsening of 30% eGFR (Restrictive Definition) - MRI/CT (Panel A) and US (Panel B) Modalities
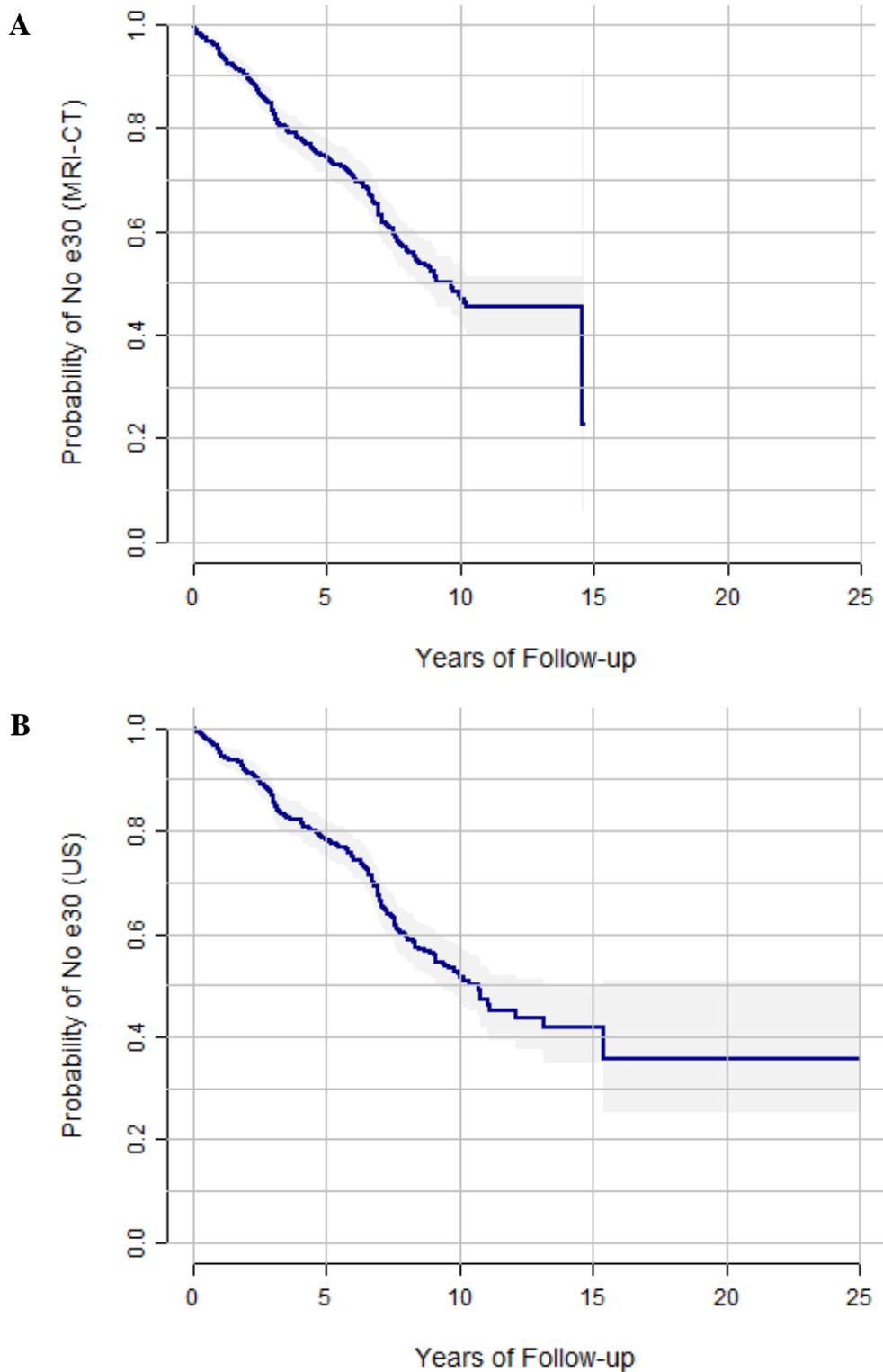
A steep decrease in the probability of no 30% worsening of eGFR was observed within five years of follow-up. The probability of no 30% worsening of eGFR at five years in the MRI/CT and US datasets

were approximately 75% and 77.5%, respectively (or conversely, a 25% and 22.5% increase in the probability of reaching 30% worsening of eGFR at five years).

For the MRI/CT dataset, a total of 1561 patients with at least one TKV measurement were available in the database. A total of 731 patients with missing covariates were excluded (of which 428 had missing baseline eGFR). After these exclusions, the analysis dataset included 830 patients of which 252 (30.4%) patients presented a 30% worsening of eGFR. The final dataset did not have missing covariates, with the exception of 295 subjects with missing genotype information. Due to the smaller sample size in the MRI/CT dataset (relative to the all modality dataset), a multivariate Cox model with simpler interaction terms was considered to avoid potential over-parameterization (see also Section 5.0). Final parameters derived with the multivariate Cox model for the MRI/CT dataset are presented in Table 23.

Table 23: Multivariate Cox Model Including Interaction Terms for the Probability of a 30% Worsening of eGFR – MRI/CT Dataset

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	P-Value
Prognostic Factors					
Ln Baseline TKV	1.5097	**4.525**	0.319216	4.73	2.30E-06
Baseline Age	0.16183	1.176	0.052673	3.07	2.10E-03
Baseline eGFR	0.03381	1.034	0.005423	6.23	4.50E-10
Interaction Terms					
eGFR:Age	-0.00114	0.999	0.000149	-7.66	1.90E-14
TKV:Age	-0.01309	0.987	0.006613	-1.98	4.80E-02

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively.

For the US dataset, a total of 1140 patients with at least one TKV measurement were available in the database. A total of 559 patients with missing covariates were removed (of which 276 had missing baseline eGFR). The final analysis dataset included 581 patients of which 220 (37.9%) presented a 30% worsening of eGFR. The final dataset did not have missing covariates, with the exception of three patients with missing height and 197 patients with missing genotype information. Due to the smaller sample size in the US dataset (relative to the all modality dataset), a multivariate Cox model with simpler interaction terms was considered to avoid potential over-parameterization (see also Section 5.0). Final parameters derived with the multivariate Cox model for the US dataset are presented in Table 24.

Table 24: Multivariate Cox Model Including Interaction Terms for the Probability of a 30% Worsening of eGFR – US Dataset

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	P-Value
Prognostic Factors					
Ln Baseline TKV	1.489258	**4.434**	0.240421	6.19	5.90E-10
Baseline Age	0.187936	1.207	0.049692	3.78	1.60E-04
Baseline eGFR	0.034134	1.035	0.004702	7.26	3.90E-13
Interaction Terms					
eGFR:Age	-0.00087	0.999	0.000154	-5.64	1.70E-08
TKV:Age	-0.01637	0.984	0.006414	-2.55	1.10E-02

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively.

The effect of ln-transformed baseline TKV in the MRI/CT and US datasets were similar, with hazard ratio [exp(Coefficient)] of 4.525 and 4.434, respectively. Likewise, effects of baseline age and baseline eGFR in the MRI/CT and US datasets were similar.

In addition, the predictive power of the above covariates in the MRI/CT and US datasets were explored based on ROC values at one and five years. Although the ROC values at year 1 for the MRI/CT dataset was slightly superior to that observed for the US dataset (0.7657 and 0.7042, respectively), ROC values at year 5 were very similar for the two datasets (0.6912 and 0.701, respectively).

Overall, the above results suggest that the effect of ln-transformed baseline TKV and predictive value in the MRI/CT dataset were similar to those observed in the US dataset.

5.1.5   30% Worsening of eGFR - Joint Model Buildup and Validation

Joint Model Buildup

A joint model linking the trajectory of TKV and the probability of a 30% worsening of eGFR was constructed as a potential drug development tool for trial enrichment. Simulations performed with the joint model may ultimately be used to select patient characteristics to predict disease progression in sub-populations of interest.

Multivariate Cox analyses have demonstrated that ln-transformed baseline TKV, baseline age, and baseline eGFR were statistically significant predictors of a 30% worsening of eGFR based on a dataset including all imaging modalities (i.e., combined MRI/CT and US measurements). Furthermore, exploratory analyses performed with the Multivariate Cox models demonstrated that results derived the MRI/CT dataset were similar to those derived with the US dataset. Based on the above results, joint modeling of TKV and the probability of avoiding a 30% worsening of eGFR ("restrictive" definition) was performed using all imaging modalities.

A total of 641 patients with at least two TKV measurements separated by at least six months were included in the analysis and a total of 3070 TKV measurements were available across subjects. A total of 192 patients presented a 30% worsening of eGFR (30.0%). Descriptive statistics of baseline characteristics of patients included in the joint modeling analysis are provided in Appendix 8.6.

The following models were tested:

TKV Model
- A linear mixed-effect model with a random intercept was used to fit ln-transformed TKV values over time

Event Model (Probability of 30% Worsening of eGFR)
- Association parameter between predicted TKV at the time of 30% worsening of eGFR was modeled using a piece-wise linear model (12 knots)
- Baseline age, baseline eGFR, and interaction terms were included

Summary results of the joint modeling analysis are presented in Table 25.

Table 25: Final Parameters of the Joint Model for the Probability of a 30% Worsening of eGFR

Parameters	Value	Standard Error	z-value	p-value
TKV Model				
Intercept	6.6684	0.0348	191.87	<0.0001
Rate of Growth	0.0516	0.0024	21.75	<0.0001
Event Model				
Association (TKV→ Event)	0.8457	0.1204	7.0233	<0.0001
Baseline eGFR	0.0101	0.0032	3.1880	0.0014
Baseline Age	0.0004	0.0077	0.0519	0.9586

The rate of growth of TKV was 0.0516 (corresponding to 5.16% per year). The association between the predicted TKV at the time of 30% worsening of eGFR (piecewise linear hazard function with 12 knots) was highly statistically significant (p<0.0001). Baseline eGFR was statistically significant (p=0.0014) while the effect of baseline age was not statistically significant (p=0.9586). Baseline age was retained in the final joint model due to the statistically significant interaction with baseline TKV and baseline eGFR previously reported in the multivariate Cox model and to allow flexibility in exploring trial enrichment strategies according to this baseline characteristic. Model adequacy was confirmed using pertinent graphics of goodness-of-fit for the longitudinal outcome (refer to Appendix 8.6).

Final Joint Model and Cross-Validation

Cross-validation was performed using a five-fold data-splitting method to evaluate the predictive performance of the final joint model. The predictive performance of the model was assessed by deriving mean prediction errors (MPE) and root-mean-square errors (RMSE) values of observed vs. predicted values of 30% worsening of eGFR. Results of the cross-validation for the probability of avoiding a 30% worsening of eGFR are presented in Table 26.

Table 26: Cross-Validation of Joint Model for the Predicted Probability of Avoiding a 30% Worsening of eGFR

Parameters	Follow-Up Times	Predicted Error Median	Predicted Error Lower	Predicted Error Upper
Bias (MPE %)	1.00	-0.03	0.51	-0.60
	3.00	0.85	1.54	-1.47
	5.00	1.19	-0.10	-0.91
	10.00	-1.67	-23.80	3.19
Precision (RMSE %)	1.00	0.68	1.57	0.17
	3.00	3.62	5.04	2.37
	5.00	5.32	7.63	4.08
	10.00	7.08	10.00	6.13

Note: Mean prediction errors (MPE) are computed as: (pred_val − obs_val)/obs_val * 100%, where obs_val and pred_val are the observed and predicted percentiles at the desired quantile (time) over the Test (or validation) group in the fold.

Root mean square errors (RMSE) are computed as: $RMSE(\%) = \left[N^{-1} \cdot \sum_{i=1}^{N} (pe_i)^2 \right]^{1/2}$

Mean prediction errors (MPE) values for avoiding a 30% worsening of eGFR over 1, 3, 5 and 10 years of follow-up were -0.03%, 0.85%, 1.19% and -1.67%, respectively. Overall, the predicted probabilities of avoiding a 30% worsening of eGFR over 10 years derived with the joint model and the prognostic factors

were within 2% of observed probabilities. The above results suggest that the ln-transformed baseline TKV, along with other prognostic factors such as baseline eGFR can accurately predict the risk of a 30% worsening of eGFR over a prolonged follow-up period.

5.1.6 30% Worsening of eGFR - Simulations

Simulations of a typical use of the biomarker in a clinical trial were performed with the final joint model to explore the effect of baseline TKV, baseline age and baseline eGFR. Results in a typical 20-year-old subject according to baseline TKV values (500, 1000, 2000, and 3000 mL) and baseline eGFR (30, 50, and 70 mL/min/1.73m^2) over different follow-times (1, 3, 5, and 10 years) are presented in Table 27.

Table 27: Predicted Probability of No 30% Worsening of eGFR in a Typical 20-year-old Subject as a Function of Baseline TKV and Baseline eGFR

Baseline Age	Baseline TKV (mL)	Follow-Up Times (Years)	Probability of No 30% Worsening of eGFR		
			eGFR 30 (mL/min/1.73m^2)	eGFR 50 (mL/min/1.73m^2)	eGFR 70 (mL/min/1.73m^2)
20	500	1	0.996	0.995	0.994
		3	**0.977**	0.971	**0.964**
		5	0.946	0.934	0.918
		10	**0.819**	0.792	**0.737**
	1000	1	0.993	0.992	0.990
		3	**0.960**	0.951	**0.938**
		5	0.910	0.891	0.863
		10	**0.731**	0.684	**0.613**
	2000	1	0.988	0.985	0.981
		3	**0.932**	0.916	**0.899**
		5	0.852	0.820	0.779
		10	**0.602**	0.532	**0.455**
	3000	1	0.984	0.979	0.974
		3	**0.907**	0.887	**0.862**
		5	0.802	0.755	0.715
		10	**0.489**	0.420	**0.349**

- Three years follow-up times in a typical 20-year-old subject

  o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 97.7%, 96.0%, 93.2% and 90.7%, respectively (or 2.3%, 4.0%, 6.8%, and 9.3% probability of <u>reaching</u> 30% worsening of eGFR).

  o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 96.4%, 93.8%, 89.9% and 86.2%, respectively (or 3.6%, 6.2%, 10.1%, and 13.8% probability of <u>reaching</u> 30% worsening of eGFR).

- 10 years follow-up times in a typical 20-year-old subject

- o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 81.9%, 73.1%, 60.2% and 48.9%, respectively (or 18.1%, 26.9%, 39.8%, and 51.1% probability of <u>reaching</u> 30% worsening of eGFR).

- o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 73.7%, 61.3%, 45.5% and 34.9%, respectively (or 26.3%, 38.7%, 54.5%, and 65.1% probability of <u>reaching</u> 30% worsening of eGFR).

The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to show a 30% worsening of eGFR during the duration of a clinical trial.

Results in a typical 40-year-old subject according to baseline TKV values (500, 1000, 2000, and 3000 mL) and over different follow-times (1, 3, 5, and 10 years) are presented in Table 28.

Table 28: Probability of No 30% Worsening of eGFR in a Typical 40-year-old Subject - Effect of Baseline TKV and Follow-Up Times

Baseline Age	Baseline TKV (mL)	Follow-Up Times (Years)	Probability of No 30% Worsening of eGFR		
			eGFR 30 (mL/min/1.73m^2)	eGFR 50 (mL/min/1.73m^2)	eGFR 70 (mL/min/1.73m^2)
40	500	1	0.995	0.994	0.993
		3	**0.968**	0.964	**0.962**
		5	0.927	0.918	0.911
		10	**0.767**	0.732	**0.709**
	1000	1	0.991	0.990	0.989
		3	**0.946**	0.941	**0.936**
		5	0.881	0.868	0.855
		10	**0.650**	0.620	**0.585**
	2000	1	0.984	0.982	0.981
		3	**0.908**	0.900	**0.892**
		5	0.801	0.786	0.772
		10	**0.486**	0.474	**0.431**
	3000	1	0.976	0.975	0.972
		3	**0.873**	0.862	**0.850**
		5	0.737	0.713	0.694
		10	**0.382**	0.336	**0.308**

- Results for three year follow-up time in a typical 40-year-old subject are summarized below:

  - o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 96.8%, 94.6%, 90.8% and 87.3%, respectively (or 3.2%, 5.4%, 9.2%, and 12.7% probability of <u>reaching</u> 30% worsening of eGFR).

  - o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and

3000 mL were 96.2%, 93.6%, 89.2% and 85.0%, respectively (or 3.8%, 6.4%, 10.8%, and 15.0% probability of <u>reaching</u> 30% worsening of eGFR).

- Results for 10 years follow-up time in a typical 40-year-old subject are summarized below:

  o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 76.7%, 65.0%, 48.6% and 38.2%, respectively (or 23.3%, 35.0%, 51.4%, and 61.8% probability of <u>reaching</u> 30% worsening of eGFR).

  o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 70.9%, 58.5%, 43.1% and 30.8%, respectively (or 29.1%, 41.5%, 56.9%, and 69.2% probability of <u>reaching</u> 30% worsening of eGFR).

The above information on baseline TKV can be used as an inclusion criterion in clinical trials to identify patients likely to show a 30% worsening of eGFR during the clinical trial.

5.2   57% Worsening of eGFR

5.2.1   57% Worsening of eGFR - Endpoint Definition and Exploratory Analyses

Based on the CDISC database, a 57% decline in eGFR relative to baseline was used to derive a binary endpoint. A subsequent measurement was required to confirm the original 57% decline in eGFR (referred as the "restrictive" definition of the endpoint). TKV values were measured by MRI, CT, or US modalities (referred to as the "All Modalities" dataset).

A total of 2355 patients with at least one TKV measurement (all modalities) in the database were available. A total of 1215 patients with missing covariates were excluded, of which 664 had missing baseline eGFR. Overall, the analysis dataset included 1140 patients with 115 (10.1%) who had a 57% worsening of eGFR. There were no missing covariates of interest in the final dataset, but there were two patients with missing height and 466 patients with missing genotype information.

After the creation of the 57% worsening of eGFR analysis dataset, the following baseline characteristics of the included patients were generated and are provided in Figure 25.

Figure 29: Baseline Characteristics of Patients included in the 57% Worsening of eGFR Analysis
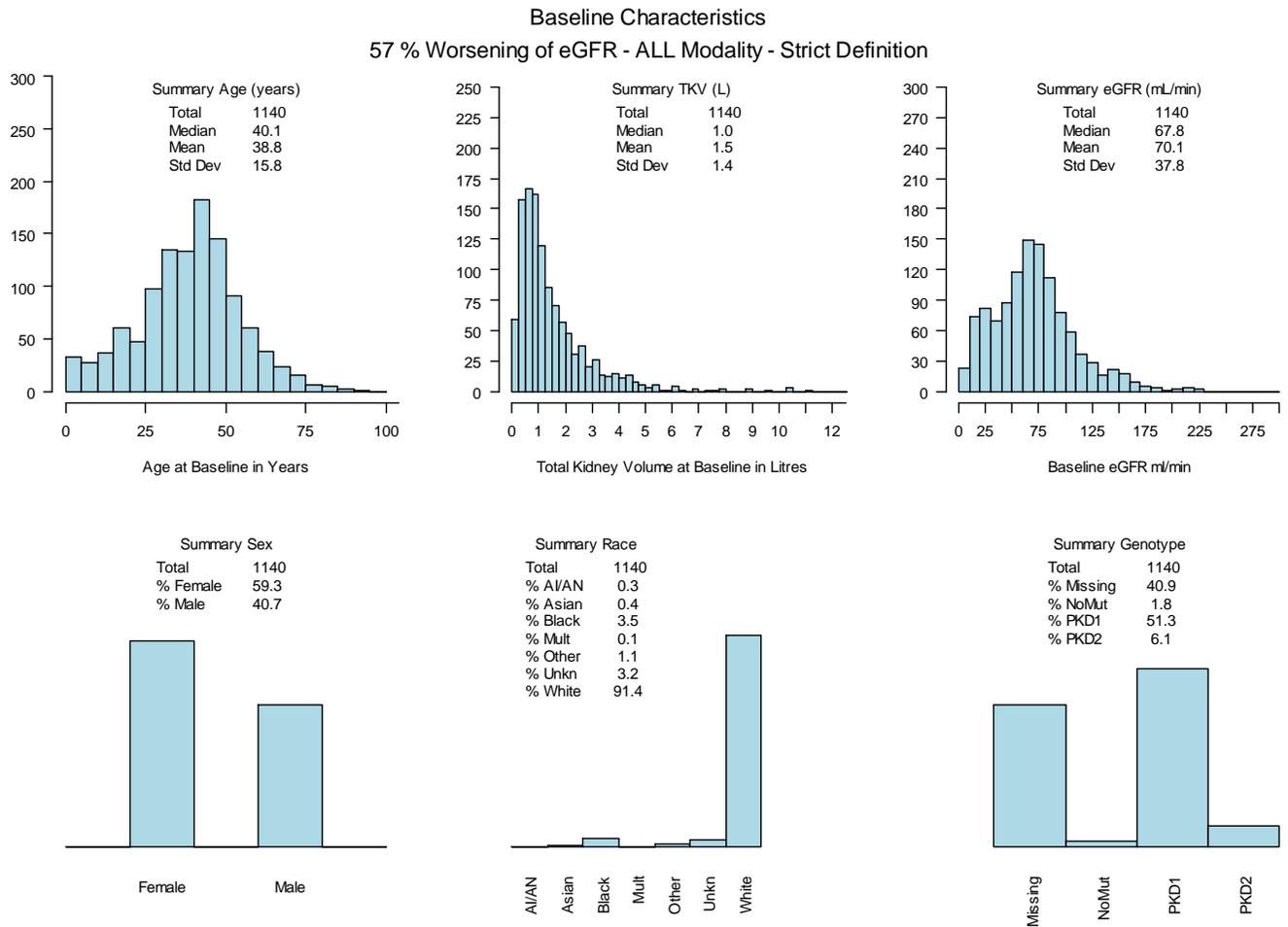
Baseline Characteristics
57 % Worsening of eGFR - ALL Modality - Strict Definition

A Kaplan-Meier figure for the probability of avoiding a 57% worsening of eGFR as a function of years of follow-up is presented in **Figure 30**.

Figure 30: Kaplan-Meier Plot for the Probability of Avoiding a 57% Worsening of eGFR as a Function of Years of Follow-Up
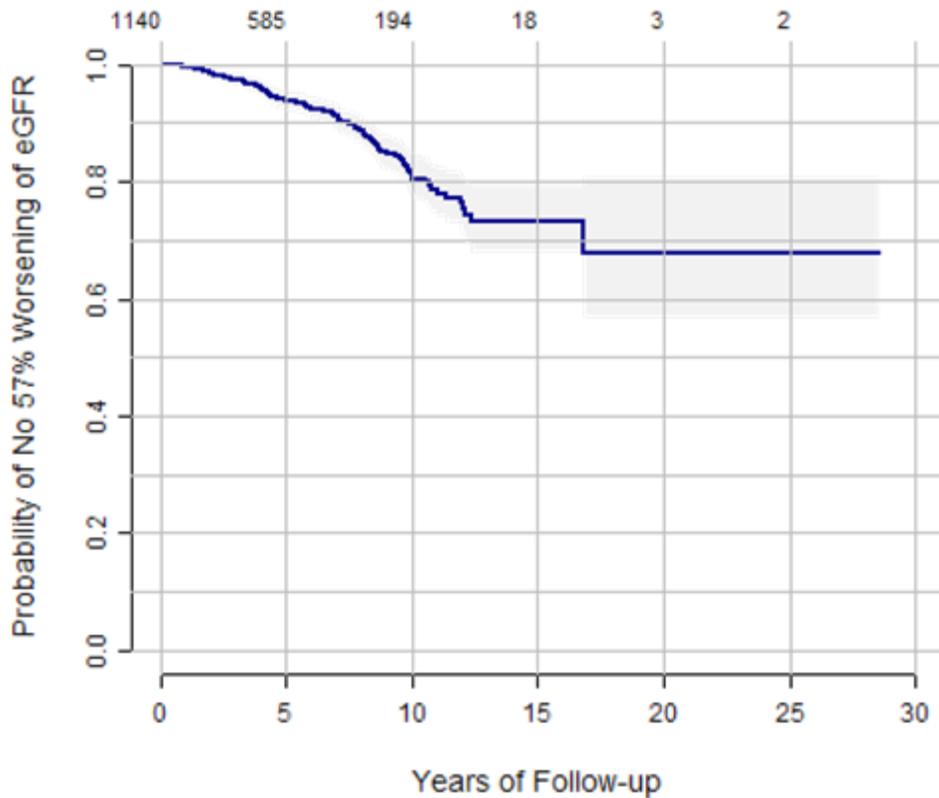
Years of follow-up were calculated relative to the first TKV measurement. A steep decrease in the probability of no 57% worsening of eGFR was observed within 5 to 10 years of follow-up. The probability of no 57% worsening of eGFR at 10 years of follow-up was approximately 80% (or conversely, a 20% increase in the probability of reaching 57% worsening of eGFR at 10 years).

A Kaplan-Meier figure for the probability of avoiding a 57% worsening of eGFR as a function of baseline TKV (< 1 or ≥1 L) and baseline eGFR (< 50 or ≥ 50 mL/min/1.73m^2) is presented in **Figure 31**.

Figure 31: Kaplan-Meier Plot for the Probability of Avoiding a 57% Worsening of eGFR as a Function of Baseline TKV and Baseline eGFR
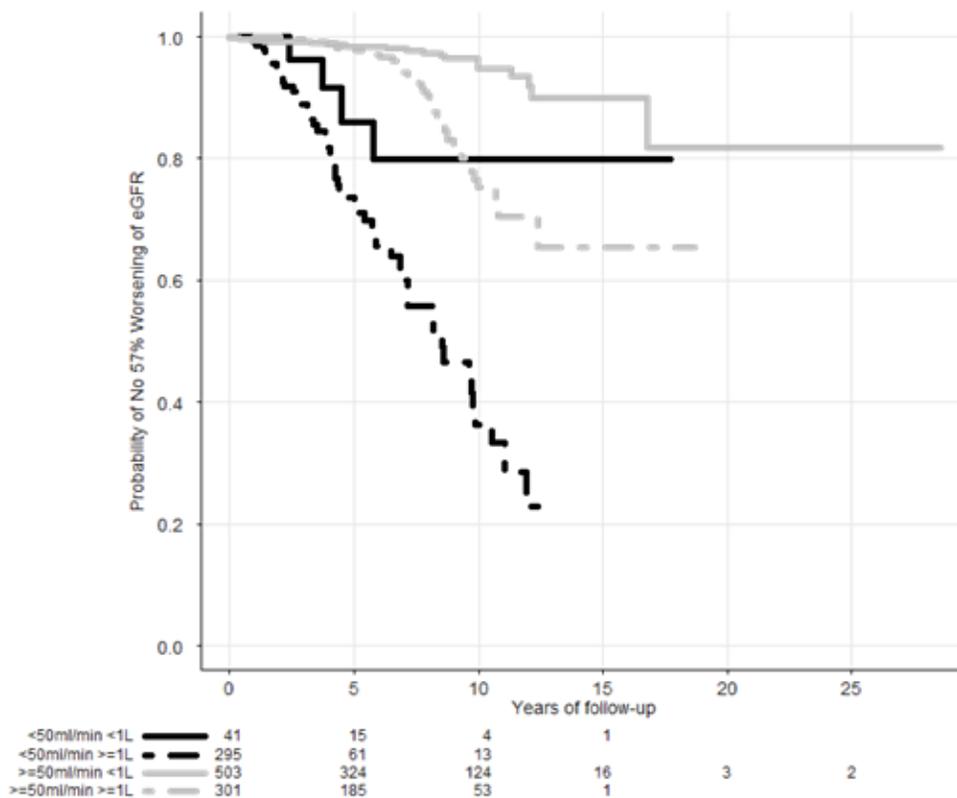
For patients with "preserved" kidney function (i.e., eGFR ≥ 50 mL/min/1.73m^2), the risk of a 57% worsening in ADPKD patients with larger TKV (≥ 1 L) was greater than that observed in patients with smaller TKV (< 1 L) (grey dashed vs. grey solid lines).

For patients with "reduced" kidney function (i.e., eGFR < 50 mL/min/1.73m^2), the risk of a 57% worsening of eGFR in ADPKD patients with larger TKV (≥ 1 L) was greater than that observed in patients with smaller TKV (< 1 L) (black dashed vs. black solid lines).

The above results suggest that TKV is prognostic for selecting patients most likely to progress to a 57% worsening of eGFR in populations with "preserved" (those mostly likely to be enrolled in a clinical trial) and "reduced" kidney function. Furthermore, the above results suggest that trial enrichment based on the selection of patient characteristics may potentially be applied to predict faster disease progression in sub-populations of interest.

A Kaplan-Meier figure for the probability of no 57% worsening of eGFR as a function of baseline TKV (< 1 or ≥1 L), baseline eGFR (< 50 or ≥ 50 mL/min/1.73m^2) and baseline age (< 40 or ≥ 40 years) is presented for information purposes in Appendix 8.7.

5.2.2   57% Worsening of eGFR - Univariate Cox Analysis

Univariate Cox models (1-by-1) were used in a first step to assess the effect of individual candidate predictors for the probability of a 57% worsening of eGFR ("restricted" definition of the endpoints). The following predictors were considered: baseline TKV (ln-transformed and untransformed), baseline height-adjusted TKV (ln-transformed and untransformed), baseline eGFR (eGFR at first TKV measurement), baseline age (age at first TKV measurement), sex, race (white and non-white), and genotype (no mutation reported, PKD1 and PKD2).

Results of the univariate Cox analysis for the probability of a 57% worsening of eGFR are presented in Table 29.

Table 29: Univariate Cox Results for the Probability of a 57% Worsening of eGFR

Covariate	N	P-Value	Sign	Hazard Ratio	Lower 95% CI	Upper 95% CI	ROC 1	ROC 5
Ln Baseline HA TKV	1138	<0.001	+	**5.40**	4.07	7.16	0.822	0.794
Ln Baseline TKV	1140	<0.001	+	**5.05**	3.82	6.68	0.817	0.793
Baseline eGFR	1140	<0.001	-	0.95	0.94	0.96	0.827	0.793
Baseline TKV	1140	<0.001	+	1.72	1.59	1.86	0.783	0.657
Baseline HA TKV	1138	<0.001	+	1.10	1.08	1.11	0.772	0.644
Baseline Age	1140	<0.001	+	1.05	1.04	1.06	0.697	0.684
Baseline Height	1138	<0.001	+	1.03	1.01	1.04	0.600	0.609
Genotype	674	0.073	+	1.52	0.66	2.43	0.542	0.538
Sex	1140	0.106	+	1.35	0.94	1.95	0.537	0.537
Race	1140	0.717	+	1.16	0.51	2.64	0.505	0.504

Notes: HA= height-adjusted; N= sample size; ROC= are under receiver operator characteristic curves for predicting the outcome at year 1 or 5. Hazard ratio for Ln Baseline HA TKV and Ln Baseline TKV were derived for a difference of 1 log (i.e., 2.7-fold increase in HA TKV and TKV). Hazard ratio for Baseline HA TKV, Baseline TKV, Baseline eGFR, Baseline Age, and Baseline Height were derived for a 1 unit increment (i.e., mL/cm, L, mL/min, year, and cm, respectively). Hazard ratio for Sex (Male vs. female comparison), Genotype (*Pkd1* vs. no mutation), and Race (white vs. non-white) were derived.

The effect of taking the natural log of baseline TKV or height-adjusted TKV was an improvement in model fit, as reflected in the higher ROC values as compared with the models based on the raw values. Because TKV growth appears to be exponential {Grantham 2006}, a log transformed change in TKV was a natural choice and it was shown to be more prognostic than a raw un-transformed TKV. Changes on a log scale reflect fold increases rather than absolute mls increase. For example, a change of 1 log would reflect a 2.718 fold increase (e.g., from 500 mls to 1359 mls or from 1000 mls to 2718 mls) and both would result in the same effect on the hazard ratio.

The effect of sex was statistically significant with a hazard ratio of 1.35 (suggesting a 35% higher probability of a 57% worsening in male patients).

All other covariates were statistically significant, with the exception of genotype (p=0.073) and race (p=0.717). Therefore, effects of genotype and race were not tested in the multivariate Cox model.

Results derived with the above univariate Cox models (1-by-1) should be interpreted with caution considering the confounding effects between covariates.

5.2.3   57% Worsening of eGFR - Multivariate Cox Analysis and Interaction Terms

In order to tease out confounding effects between baseline TKV and other covariates such as baseline age and baseline eGFR, a multivariate Cox analysis was performed for the probability of a 57% worsening of eGFR ("restricted" definition of the endpoints). Although ln-transformed baseline height-adjusted TKV and ln-transformed baseline TKV resulted in a similar predictive power (ROC values at one and five years, refer to **Table 29**), ln-transformed baseline TKV was used in the multivariate Cox analysis since it was deemed more convenient to use and to derive in a clinical setting. As ln-transformed baseline TKV was our key predictor of interest, it was the first variable incorporated in the multivariate Cox model, and it was included in all subsequent models. Other covariates and interaction terms were tested through forward stepwise model building. Final parameters included in the multivariate Cox model are presented in **Table 30.**

Table 30: Final Multivariate Cox Model Including Interaction Terms for the Probability of a 57% Worsening of eGFR

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	P-Value
Prognostic Factors					
Ln Baseline TKV	2.317417	10.14942	0.553168	4.19	2.80E-05
Baseline Age	0.234893	1.264773	0.095326	2.46	0.014
Baseline eGFR	0.005083	1.005096	0.014457	0.35	0.725
Interaction Terms					
eGFR:Age	-0.00089	0.99911	0.000356	-2.5	0.012
TKV:Age	-0.02516	0.975157	0.01158	-2.17	0.03

As shown in Table 30, the association of TKV with a 57% decline in eGFR is highly statistically significant, independent of age and eGFR. In addition to ln-transformed baseline TKV, the effect of baseline age was significant (P<0.05). It is well known that TKV, age, and eGFR are not completely independent. Therefore, the following interaction terms were tested in the multivariate Cox model: 1) interaction between ln-transformed baseline TKV and baseline age, and 2) interaction between baseline eGFR and baseline age. This interaction model resulted in the highest ROC1 and ROC5 values (0.8705 and 0.8320, respectively). Stepwise results of the multivariate Cox analysis, as well as different interaction models tested presented in Appendix 8.7.

Based on ROC values (predictive value of model) as well as Z and p-values (statistical contribution of individual covariates), these results suggest that ln-transformed baseline TKV is the most important prognostic biomarker of progression to 57% worsening of eGFR, though age and eGFR also contribute independent predictive information.

Overall, the above results suggest that ln-transformed baseline TKV was the most important prognostic biomarker of disease progression in ADPKD patients. The above information on baseline TKV can be

used as an inclusion criterion in clinical trials to identify patients likely to show a 57% worsening of eGFR during a clinical trial.

An exploratory full-model analysis was performed to assess whether the prognostic value of ln-transformed baseline TKV was preserved after forcing other non-significant covariates into the final multivariate Cox model. After including the effect of sex (p=0.74) and race (p=0.58) in the multivariate Cox model, the p-value associated to ln-transformed baseline TKV remained highly significant (P<0.001) (refer to Appendix 8.7). The effect of gender was not statistically significant in either the univariate or multivariate Cox model.

Likewise, the effect of genotype on PKD is mediated by its effects on cyst number and growth. Therefore, genotyping (i.e., PKD1 vs. PKD2) adds little to imaging of TKV as a biomarker for predicting the natural history of the disease in individual cases.

Hazard ratios derived with the final multivariate Cox model are presented in Table 31.

Table 31: Hazard Ratios for the Probability of a 57% Worsening of eGFR

Parameters	Hazard Ratio	Lower 95% CI	Upper 95% CI
Prognostic Factors			
Ln Baseline TKV	10.149	3.432	30.012
Baseline Age	1.265	1.049	1.525
Baseline eGFR	1.005	0.977	1.034
Interaction Terms			
TKV:Age	0.975	0.953	0.998
eGFR:Age	0.999	0.998	1.000

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively.

For each increase of 1 log TKV (i.e. TKV 1000 mls vs. 2738 mls), holding age and eGFR constant, there is an approximate 10-fold increase in the probability of a 57% worsening of eGFR. Baseline age and baseline eGFR were associated with hazard ratios of 1.265 and 1.005, respectively. These hazard ratios were derived assuming that other prognostic factors were kept constant, in addition to ignoring interaction terms.

A sensitivity analysis was performed based on a dataset in which a 57% worsening of eGFR was defined as a 57% decline in eGFR relative to the baseline without the need of a subsequent confirmatory measurement (referred as the "non-restrictive endpoint"). This sensitivity analysis was performed to examine potential difference in outcome between the "restrictive" and "non-restrictive" definition of a 57% worsening of eGFR endpoints. Statistical outputs are provided in Appendix 8.7. Results of the exploratory analysis are summarized below.

- There were 2355 patients with at least one TKV measurement in the database. A total of 1203 patients with insufficient information were excluded, of which 664 had missing baseline eGFR. Overall the analysis dataset included 1152 patients of which 194 (16.8%) progressed to a 57% worsening of eGFR events. There was no missing covariate information except for two patients with missing heights and 471 with missing genotypes.

- Hazard ratios for the effect of ln-transformed baseline TKV, baseline age and baseline eGFR derived with the "non-restrictive" definition of a 57% worsening of eGFR (7.424, 1.245 and 1.018, respectively) were consistent with those derived with the "restrictive" definition (10.149, 1.265 and 1.005, respectively).

5.2.4   57% Worsening of eGFR - Exploratory Analyses Based on MRI/CT and US Modalities

Based on the request from the regulatory agencies to verify equivalence of the imaging modalities, each endpoint dataset was divided into two separate datasets (MRI/CT and US) to perform a Cox analysis. It is important to emphasize that the two modality datasets do not reflect a comparison of the same subjects using different modalities, but in fact are primarily different subject populations. Critical characteristics of these two subsets differ with regard to age and kidney function, where the MRI/CT subgroup is older and has lower kidney function or more progressive renal insufficiency. In addition, the MRI/CT and US modality datasets are smaller in sample size relative to the "all modality" dataset (i.e., a combination of all modalities).

Exploratory analyses were performed based on a dataset including TKV values measured with MRI and CT vs. US for a 57% worsening of eGFR ("restrictive" definition). This exploratory analysis was performed to examine potential differences in outcome between the "MRI-CT" dataset relative to the "US" dataset. Similar analyses as described in Section 5.2.3 were performed. Summary results are presented in Appendix 8.7. Kaplan-Meier figures for the probability of avoiding a 57% worsening of eGFR for the MRI/CT and US datasets are presented in **Figure 32**.

Figure 32: Kaplan-Meier Plots for the Probability of No Worsening of 57% eGFR (Restrictive Definition) - MRI/CT (Panel A) and US (Panel B) Modalities
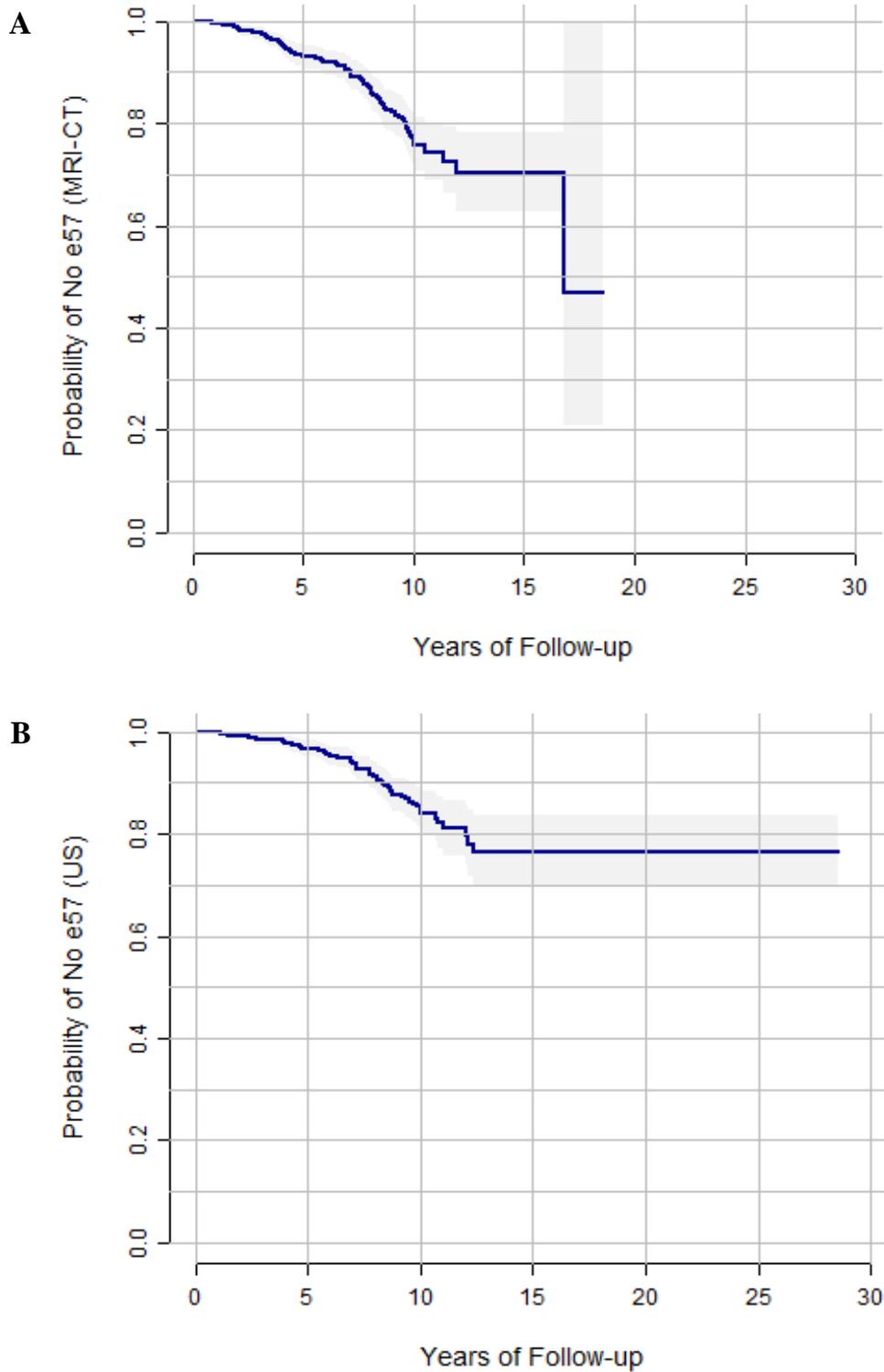
A decrease in the probability of no 57% worsening of eGFR was observed within 5 to 10 years of follow-up. The probability of no 57% worsening of eGFR at 10 years for the MRI/CT and US datasets

were approximately 75% and 82.5%, respectively (or conversely, a 25% and 17.5% increase in the probability of reaching 57% worsening of eGFR at 10 years).

For the MRI/CT dataset, a total of 1561 patients with at least one TKV measurement were available in the database. A total of 731 patients with missing covariates were removed (of which 428 had missing baseline eGFR. The final analysis dataset included 830 patients in which a total of 252 (30.4%) patients presented a 57% worsening of eGFR. The final dataset did not have missing covariates, with the exception of 295 subjects with missing genotype information. Final parameters derived with the multivariate Cox model for the MRI/CT dataset are presented in Table 32.

Table 32: Multivariate Cox Model Including Interaction Terms for the Probability of a 57% Worsening of eGFR – MRI/CT Modalities

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	p-value
Prognostic Factors					
Ln Baseline TKV	2.7896	**16.275**	0.716973	3.891	0.0001
Baseline Age	0.2695	1.309	0.123732	2.178	0.029
Baseline eGFR	0.0153	1.015	0.016392	0.936	0.350
Interaction Terms					
eGFR:Age	-0.0011	0.999	0.000405	-2.73	0.0063
TKV:Age	-0.0285	0.972	0.014618	-1.948	0.051

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively.

For the US dataset, a total of 1140 patients with at least one TKV measurement were available in the database. A total of 559 patients with missing covariates were removed (of which 276 had missing baseline eGFR). The analysis dataset included 581 patients in which a total of 220 (37.9%) patients presented a 57% worsening of eGFR. The final dataset did not have missing covariates, with the exception of three patients with missing height and 197 patients with missing genotype information. Final parameters derived with the multivariate Cox model for the US dataset are presented in Table 33.

Table 33: Multivariate Cox Model Including Interaction Terms for the Probability of a 57% Worsening of eGFR – US Modality

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	p-value
Prognostic Factors					
Ln Baseline TKV	2.714721	**15.100**	0.786772	3.4505	0.00056
Baseline Age	0.348621	1.417	0.141213	2.4688	0.014
Baseline eGFR	0.001185	1.001	0.019063	0.0622	0.950
Interaction Terms					
eGFR:Age	-0.00094	0.999	0.000501	-1.8662	0.062
TKV:Age	-0.04145	0.959	0.019012	-2.1802	0.029

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively.

The effect of ln-transformed baseline TKV in the MRI/CT and US datasets were similar, with hazard ratio [exp(Coefficient)] of 16.275 and 15.100, respectively. Likewise, effects of baseline age and baseline eGFR in the MRI/CT and US datasets were similar.

In addition, the predictive power of the above covariates in the MRI/CT and US datasets were explored based on ROC values at one and five years. For the MRI/CT and US datasets, ROC values at one year (0.8852 and 0.8687, respectively) and five years (0.8369 and 0.8307, respectively) were very similar.

Overall, statistical results derived with the MRI/CT and US datasets were very similar.

5.2.5   57% Worsening of eGFR - Joint Model Buildup and Validation

Joint Model Buildup

A joint model linking the trajectory of TKV and the probability of a 57% worsening of eGFR was constructed as a potential drug development tool for trial enrichment. Simulations performed with the joint model may ultimately be used to select patient characteristics to predict disease progression in sub-populations of interest.

Multivariate Cox analyses have demonstrated that ln-transformed baseline TKV and baseline age were statistically significant predictors for the probability of a 57% worsening of eGFR ("restricted") based on a dataset including all imaging modalities (i.e., combined MRI/CT and US measurements). Furthermore, exploratory analyses performed with the Multivariate Cox models demonstrated that results derived from the MRI/CT dataset were similar to those derived with the US dataset. Based on the above results, joint modeling of TKV and the probability of avoiding a 57% worsening of eGFR ("restrictive") was performed using all imaging modalities.

A total of 703 patients with at least two TKV measurements separated by at least six months were included in the analysis. A total of 2857 TKV measurements were available across subjects. A total of 59 patients presented a 57% worsening of eGFR (8.4%). Descriptive statistics of baseline characteristics of patients included in the joint modeling analysis are provided in Appendix 8.7.

The following models were tested:

> **TKV Model**
> - A linear mixed-effect model with a random intercept was used to fit ln-transformed TKV values over time

> **Event Model (Probability of 57% Worsening of eGFR)**
> - Association parameter between predicted TKV at the time of 57% worsening of eGFR was modeled using a piece-wise linear model (12 knots)
> - Baseline eGFR, baseline age, and interaction terms were tested

Summary results of the joint modeling analysis are presented in Table 34.

Table 34: Final Parameters of the Joint Model for the Probability of 57% Worsening of eGFR

Parameters	Value	Standard Error	z-value	p-value
TKV Model				
Intercept	6.6903	0.0268	249.31	<0.0001
Rate of Growth	0.0538	0.0022	24.31	<0.0001
Event Model				
Association (TKV→ Event)	1.4902	0.2526	5.8998	<0.0001
Baseline eGFR	-0.0275	0.0083	-3.3172	0.0009
Baseline Age	0.0024	0.0146	0.1632	0.8704

The rate of growth of TKV was 0.0538 (corresponding to 5.38% growth per year). The association between the predicted TKV at the time of 57% worsening of eGFR (piecewise linear hazard function) was highly statistically significant (p<0.0001). Baseline eGFR was statistically significant (p=0.0009). The effect of baseline age was not statistically significant (p=0.8704). Baseline age was retained in the final joint model due to the statistically significant interaction with baseline TKV and baseline eGFR previously reported in the multivariate Cox model and to allow flexibility in exploring trial enrichment strategies according to this baseline characteristic. Model adequacy was confirmed using pertinent graphics of goodness-of-fit for the longitudinal outcome (refer to Appendix 8.7).

Cross-Validation of Joint Model

Cross-validation was performed using a 10-fold data splitting method to evaluate the predictive performance of the final joint model. The predictive performance of the joint model was assessed by deriving mean prediction errors (MPE) and root mean square errors (RMSE) values for avoiding a 57% worsening of eGFR. Results of the cross-validation are presented in Table 35.

Table 35: Cross-Validation of Joint Model for the Predicted Probability of Avoiding a 57% Worsening of eGFR

Parameters	Follow-Up Times	Predicted Error Median	Predicted Error Lower	Predicted Error Upper
Bias (MPE %)	1.00	-0.06	-0.24	-0.02
	3.00	0.52	1.18	-0.07
	5.00	1.78	4.07	-0.29
	10.00	5.11	-4.74	-0.74
Precision (RMSE %)	1.00	0.01	0.06	0.00
	3.00	1.13	2.81	0.01
	5.00	2.31	4.30	0.08
	10.00	5.91	13.30	1.71

Note: Mean prediction errors (MPE) are computed as: (pred_val – obs_val)/obs_val * 100%, where obs_val and pred_val are the observed and predicted percentiles at the desired quantile (time) over the Test (or validation) group in the fold. Root mean square errors (RMSE) are computed as: $RMSE(\%) = \left[N^{-1} \cdot \sum_{i=1}^{N} (pe_i)^2 \right]^{1/2}$

Mean prediction errors (MPE) values for avoiding a 57% worsening of eGFR over 1, 3, 5 and 10 years of follow-up were -0.06%, 0.52%, 1.78% and 5.11%, respectively. The predicted probabilities of avoiding a 57% worsening of eGFR over 10 years derived with the joint model and the prognostic factors in ADPKD patients were approximately within 5% of observed probabilities. Assuming an 80% probability of

avoiding a 57% worsening of eGFR at 10 years of follow-up, the predicted error would be approximately 4% (i.e., 80% x 0.05).

The above results suggest that the joint model, along with TKV and other prognostic factor such as baseline age and baseline eGFR can accurately predict the risk of a 57% worsening of eGFR over a prolonged follow-up period.

5.2.6   57% Worsening of eGFR - Simulations

Simulations were performed with the final joint model to explore the effect of baseline TKV, baseline age and baseline eGFR. Results in a typical 20-year-old subject according to baseline TKV values (500, 1000, 2000, and 3000 mL) and baseline eGFR (30, 50, and 70 mL/min/1.73m^2) over different follow-up times (1, 3, 5, and 10 years) are presented in Table 36.

Table 36: Joint Model – Predicted Probability of No 57% Worsening of eGFR in a Typical 20-year-old Subject as a Function of Baseline TKV and Baseline eGFR

Baseline Age	Baseline TKV (mL)	Follow-Up Times (Years)	Probability of No 57% Worsening of eGFR		
			eGFR 30 mL/min	eGFR 50 mL/min	eGFR 70 mL/min
20	500	1	1.000	1.000	1.000
		3	**0.995**	0.997	**0.998**
		5	0.982	0.989	0.994
		10	0.641	0.792	0.878
	1000	1	0.999	1.000	1.000
		3	**0.986**	0.992	**0.995**
		5	0.954	0.975	0.983
		10	0.387	0.599	0.742
	2000	1	0.998	0.999	0.999
		3	**0.966**	0.980	**0.989**
		5	0.902	0.935	0.962
		10	0.122	0.350	0.398
	3000	1	0.997	0.998	0.999
		3	**0.943**	0.964	**0.981**
		5	0.838	0.895	0.938
		10	0.030	0.119	0.321

- Results for three years follow-up times in a typical 20-year-old subject are summarized below:

  o For a typical baseline eGFR of 30 mL/min/1.73m2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 95.5%, 98.6%, 96.6% and 94.3%, respectively respectively (or 4.5%, 1.4%, 3.4%, and 5.7% probability of <u>reaching</u> 57% worsening of eGFR).

  o For a typical baseline eGFR of 70 mL/min/1.73m2, mean predicted probabilities of avoiding a 30% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and

3000 mL were 99.8%, 99.5%, 98.9% and 98.1%, respectively respectively (or 0.2%, 0.5%, 1.1%, and 1.9% probability of <u>reaching</u> 57% worsening of eGFR).

- Results for 10 years follow-up times in a typical 20-year-old subject are summarized below:

  o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 64.1%, 38.7%, 12.2% and 3.0%, respectively respectively (or 35.9%, 61.3%, 87.8%, and 97.0% probability of <u>reaching</u> 57% worsening of eGFR).

  o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 87.8%, 74.2%, 39.8% and 32.1%, respectively respectively (or 12.2%, 25.8%, 60.2%, and 67.9% probability of <u>reaching</u> 57% worsening of eGFR).

The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to show a 57% worsening of eGFR during the duration of a clinical trial.

Simulation results in a typical 40-year-old subject according to baseline TKV values (500, 1000, 2000, and 3000 mL) and baseline eGFR (30, 50, and 70 mL/min/1.73m^2) over different follow-up times (1, 3, 5, and 10 years) are presented in Table 37.

Table 37: Joint Model – Predicted Probability of No 57% Worsening of eGFR in a Typical 40-year-old Subject as a Function of Baseline TKV and Baseline eGFR

Baseline Age	Baseline TKV (mL)	Follow-Up Times (Years)	Probability of No 57% Worsening of eGFR		
			eGFR 30 (mL/min/1.73m^2)	eGFR 50 (mL/min/1.73m^2)	eGFR 70 (mL/min/1.73m^2)
40	500	1	1.000	1.000	1.000
		3	**0.994**	0.996	**0.998**
		5	0.980	0.988	0.993
		10	0.648	0.811	0.883
	1000	1	0.999	1.000	1.000
		3	**0.985**	0.991	**0.995**
		5	0.951	0.972	0.984
		10	0.429	0.581	0.755
	2000	1	0.998	0.999	0.999
		3	**0.961**	0.977	**0.989**
		5	0.890	0.929	0.963
		10	0.091	0.320	0.534
	3000	1	0.996	0.998	0.999
		3	**0.935**	0.963	**0.977**
		5	0.812	0.890	0.931
		10	0.065	0.243	0.373

- Three years follow-up times in a typical 40-year-old subject

    - For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 99.4%, 98.5%, 96.1% and 93.5%, respectively (or 0.6%, 1.5%, 3.9%, and 6.5% probability of <u>reaching</u> 57% worsening of eGFR).

    - For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 99.8%, 99.5%, 98.9% and 97.7%, respectively (or 0.2%, 0.5%, 1.1%, and 2.3% probability of <u>reaching</u> 57% worsening of eGFR).

- 10 years follow-up times in a typical 40-year-old subject

    - For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 64.8%, 42.9%, 9.1% and 6.5%, respectively (or 35.2%, 57.1%, 90.9%, and 93.5% probability of <u>reaching</u> 57% worsening of eGFR).

    - For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding a 57% worsening of eGFR for baseline TKV values of 500, 1000, 2000 and 3000 mL were 88.3%, 75.5%, 53.4% and 37.3%, respectively (or 11.8%, 24.5%, 46.6%, and 62.7% probability of <u>reaching</u> 57% worsening of eGFR).

The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to show a 57% worsening of eGFR during the duration of a clinical trial.

5.3   ESRD

5.3.1   ESRD - Endpoint Definition and Exploratory Analysis

Information on ESRD was specifically provided in the CDISC database. If no information of ESRD was available up to the date of last follow-up, the patient was not deemed to have progressed to ESRD. TKV values measured by MRI, CT, or US modalities were used (referred as the "All Modalities" dataset).

A total of 2355 patients with at least one TKV measurement (all modalities) were available in the database. A total of 736 patients with missing covariates were excluded (of which a total of 664 had missing baseline eGFR). Overall, the analysis dataset included 1619 patients of which 354 (21.9%) had ESRD. There were no missing covariates of interest in the final dataset, but there were five patients with missing height and 781 patients with missing genotype information.

After the creation of the ESRD analysis dataset, the following baseline characteristics of the included patients were generated and are provided in Figure 25.

Figure 33: Baseline Characteristics of Patients included in the ESRD Analysis
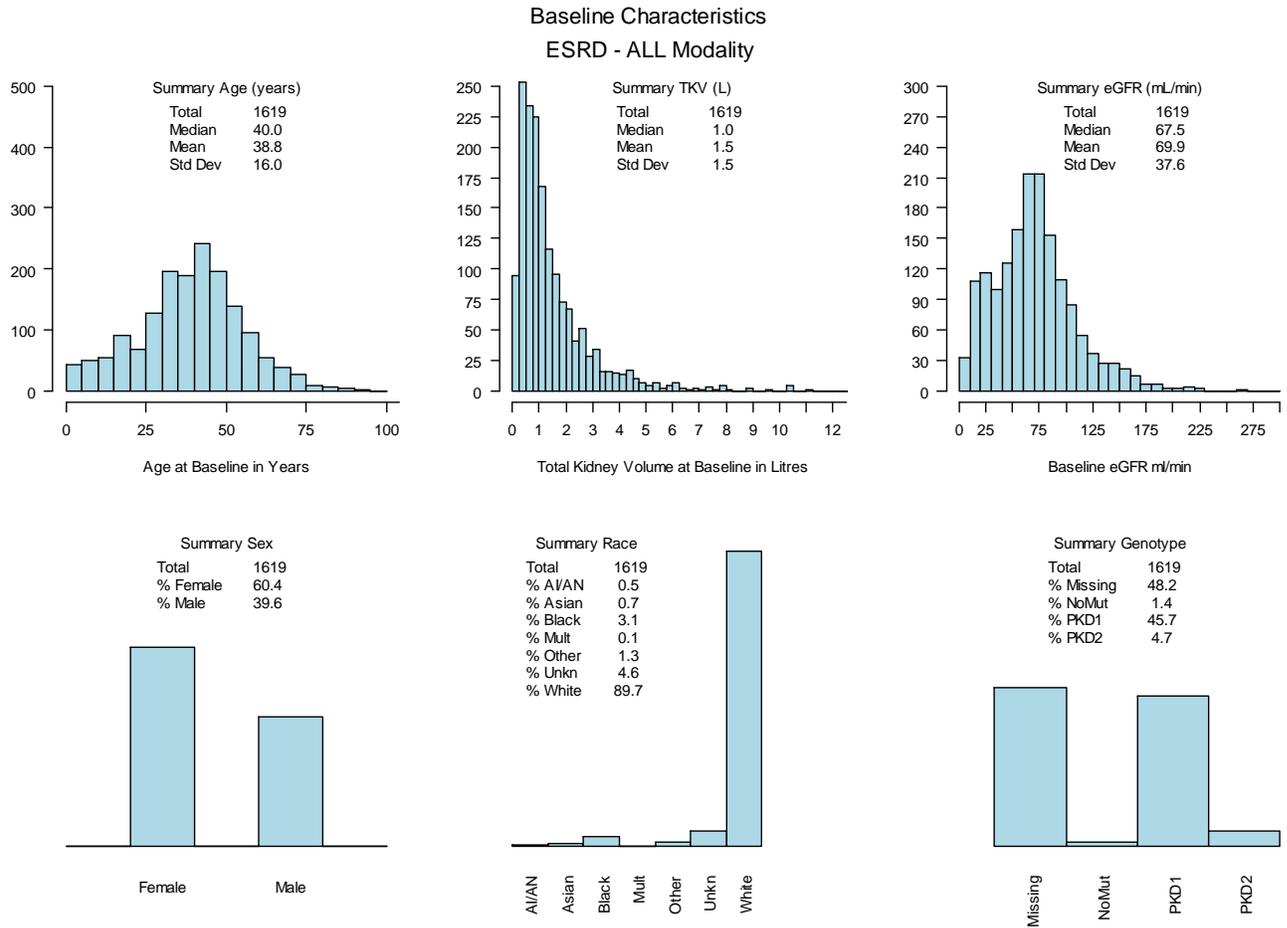
Baseline Characteristics
ESRD - ALL Modality

A Kaplan-Meier figure for the probability of avoiding ESRD as a function of years of follow-up is presented in Figure 34.

Figure 34: Kaplan-Meier Plot for the Probability of Avoiding ESRD as a Function of Years of Follow-Up
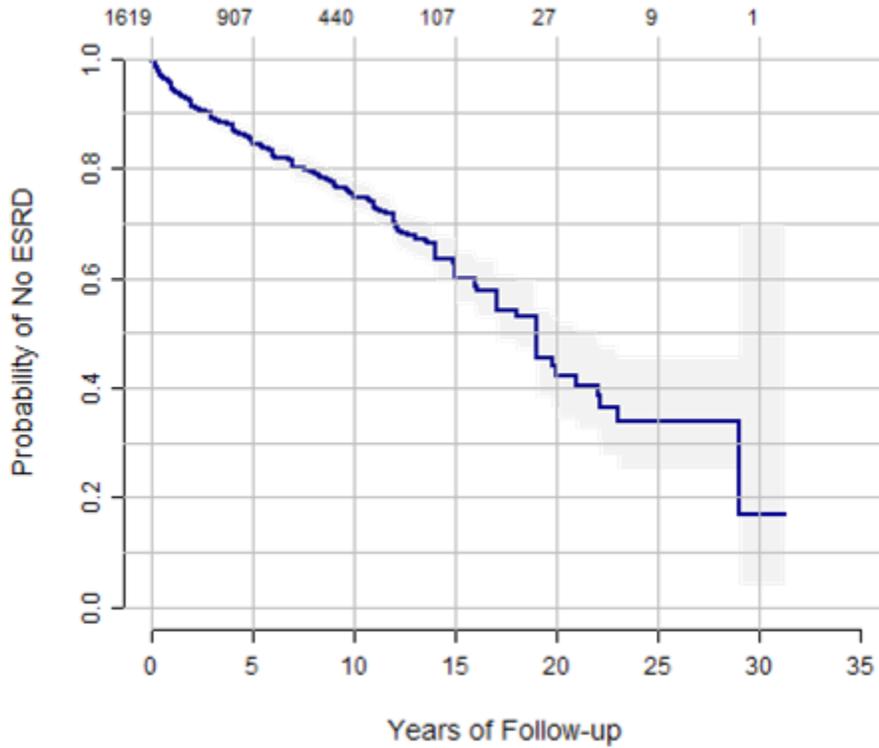
Years of follow-up were calculated relative to the first TKV measurement. A steep decrease in the probability of avoiding ESRD was observed within 10 years of follow-up. The probability of no ESRD at 10 years of follow-up was approximately 75% (or conversely, a 25% increase in the probability of reaching ESRD at 10 years).

A Kaplan-Meier figure for the probability of avoiding ESRD as a function of baseline TKV (< 1 or ≥ 1 L) and baseline eGFR (< 50 or ≥ 50 mL/min/1.73m^2) is presented in **Figure 35**.

Figure 35: Kaplan-Meier Plot for the Probability of Avoiding ESRD as a Function of Baseline TKV and Baseline eGFR

For patients with "preserved" kidney function (i.e., eGFR ≥ 50 mL/min/1.73m^2), the risk of ESRD in ADPKD patients with larger TKV (≥ 1 L) was greater than that observed in patients with smaller TKV (< 1 L) (grey dashed vs. grey solid lines).

For patients with "reduced" kidney function (i.e., eGFR < 50 mL/min/1.73m^2), the risk of ESRD in ADPKD patients with larger TKV (≥ 1 L) was greater than that observed in patients with smaller TKV (< 1 L) (black dashed vs. black solid lines).

The above results suggest that TKV is prognostic for selecting patients most likely to progress to ESRD in populations with "preserved" (those mostly likely to be enrolled in a clinical trial) and "reduced" kidney function. Furthermore, the above results suggest that trial enrichment based on the selection of patient characteristics may potentially be applied to predict faster disease progression.

A Kaplan-Meier figure for the probability of no ESRD as a function of baseline TKV (< 1 or ≥ 1 L), baseline eGFR (< 50 or ≥ 50 mL/min/1.73m^2) and baseline age (< 40 or ≥ 40 years) is presented for information purposes in Appendix 8.8.

5.3.2   ESRD - Univariate Cox Analysis

Univariate Cox models (1-by-1) were used in a first step to assess the effect of individual candidate predictors for the probability of ESRD. The following predictors were considered: baseline TKV (ln-transformed and untransformed), baseline height-adjusted TKV (ln-transformed and untransformed), baseline eGFR (eGFR at first TKV measurement), baseline age (age at first TKV measurement), sex, race (white and non-white), and genotype (no mutation reported, PKD1 and PKD2).

Results of the univariate Cox analysis for the probability of ESRD are presented in Table 38.

Table 38: Univariate Cox Results for the Probability of ESRD

Covariate	N	P-Value	Sign	Hazard Ratio	Lower 95% CI	Upper 95% CI	ROC 1	ROC 5
Baseline eGFR	1619	<0.001	-	0.90	0.89	0.91	0.943	0.933
Ln Baseline HA TKV	1614	<0.001	+	**5.45**	4.72	6.29	0.834	0.805
Ln Baseline TKV	1619	<0.001	+	**5.17**	4.49	5.96	0.832	0.805
Baseline HA TKV	1614	<0.001	+	1.07	1.07	1.08	0.714	0.625
Baseline TKV	1619	<0.001	+	1.49	1.44	1.54	0.715	0.621
Baseline Age	1619	<0.001	+	1.05	1.05	1.06	0.713	0.712
Baseline Height	1614	<0.001	+	1.03	1.02	1.03	0.592	0.597
Sex	1619	<0.001	+	1.47	1.19	1.81	0.547	0.547
Genotype	838	0.064	+	1.08	0.93	2.18	0.528	0.526
Race	1619	0.899	-	0.97	0.65	1.46	0.501	0.501

Notes: HA= height-adjusted; N= sample size; ROC= are under receiver operator characteristic curves for predicting the outcome at year 1 or 5. Hazard ratio for Ln Baseline HA TKV and Ln Baseline TKV were derived for a difference of 1 log (i.e., 2.7-fold increase in HA TKV and TKV). Hazard ratio for Baseline HA TKV, Baseline TKV, Baseline eGFR, Baseline Age, and Baseline Height were derived for a 1 unit increment (i.e., mL/cm, L, mL/min, year, and cm, respectively). Hazard ratio for Sex (Male vs. female comparison), Genotype (PKD1 vs. no mutation), and Race (white vs. non-white) were derived.

The effect of baseline eGFR was statistically significant, with ROC values at one and five years of 0.943 and 0.933, respectively. The effect of taking the natural log of baseline TKV or height-adjusted TKV was an improvement in model fit, as reflected in the higher ROC values as compared with the models based on the raw values. Because TKV growth appears to be exponential {Grantham 2006}, a log transformed change in TKV was a natural choice and it was shown to be more prognostic than a raw un-transformed TKV. Changes on a log scale reflect fold increases rather than absolute mls increase. For example, a change of 1 log would reflect a 2.718 fold increase (e.g., from 500 mls to 1359 mls or from 1000 mls to 2718 mls) and both would result in the same effect on the hazard ratio.

The effect of sex was statistically significant with a hazard ratio of 1.47 (suggesting a 47% higher probability of ESRD in male patients). The effect of gender was not statistically significant in the

multivariate Cox due to the association between body size and gender (i.e., men generally have larger kidneys than women).

The other covariates were statistically significant, with the exception of genotype (p=0.064) and race (p=0.899). Therefore, effects of genotype and race were not tested in the multivariate Cox model.

The effect of genotype on PKD is mediated by its effects on cyst number and growth. Therefore, genotyping (i.e., PKD1 vs. PKD2) adds little to imaging of TKV as a biomarker for predicting the natural history of the disease in individual cases.

Results derived with the above univariate Cox models (1-by-1) should be interpreted with caution considering the confounding effects between covariates such as baseline eGFR and baseline TKV.

5.3.3   ESRD - Multivariate Cox Analysis and Interaction Terms

In order to tease out confounding effects between ln-transformed baseline TKV and other covariates such as baseline age and baseline eGFR, a multivariate Cox analysis was performed and interaction terms were included in the model. Although ln-transformed baseline height-adjusted TKV and ln-transformed baseline TKV resulted in a similar predictive power (ROC values at one and five years) based on the univariate Cox analysis (refer to **Table 38**), ln-transformed baseline TKV was used in the multivariate Cox analysis since it was deemed more convenient to use in a clinical setting. As ln-transformed baseline TKV was our key predictor of interest, it was the first variable incorporated in the multivariate Cox model, and it was included in all subsequent models. Other covariates and interaction terms were tested through stepwise model building. Final parameters included in the multivariate Cox model and statistically significant interaction terms are presented in Table 39.

Table 39: Final Multivariate Cox Model Including Interaction Terms for the Probability of ESRD

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	P-Value
Prognostic Factors					
Ln Baseline TKV	1.502374	4.492341	0.296506	5.07	4.00E-07
Baseline Age	0.172868	1.188709	0.045862	3.77	0.00016
Baseline eGFR	-0.027529	0.972846	0.010681	-2.58	0.00995
Interaction Terms					
TKV:Age	-0.018000	0.982161	0.005535	-3.25	0.00115
eGFR:Age	-0.001687	0.998315	0.000242	-6.98	2.90E-12

As shown in Table 39, the association of TKV with ESRD is highly statistically significant, independent of age and eGFR. In addition to ln-transformed baseline TKV, the effect of baseline age and baseline eGFR were statistically significant. It is well known that TKV, age, and eGFR are not completely independent. Therefore, the following interaction terms were tested in the multivariate Cox model: 1) interaction between ln-transformed baseline TKV and baseline age, and 2) interaction between baseline eGFR and baseline age. The final model including the interaction term resulted in the highest ROC1 and ROC5 values (0.9519 and 0.9394, respectively). Stepwise results of the multivariate Cox analysis, as well as different interactions models tested are presented in Appendix 8.8.

Based on ROC values (predictive value of model) as well as Z and p-values (statistical contribution of individual covariates), these results suggest that ln-transformed baseline TKV is the most important prognostic biomarker of progression to ESRD, though age and eGFR also contribute independent predictive information.

The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to progress to ESRD during a clinical trial. Other prognostic factors such as baseline age and baseline eGFR may be considered.

An exploratory full model analysis was performed to assess whether the prognostic value of ln-transformed baseline TKV was preserved after forcing other non-significant covariates into the final multivariate Cox model. After including the effect of sex (p=0.3609) and race (p=0.7326) in the multivariate Cox model, the p-value associated to ln-transformed baseline TKV remained highly significant (P<0.001) (refer to Appendix 8.8).

Hazard ratios derived with the final multivariate Cox model are presented in Table 40.

Table 40: Hazard Ratios for the Probability of ESRD

Parameters	Hazard Ratio	Lower 95% CI	Upper 95% CI
Prognostic Factors			
Ln Baseline TKV	4.492	2.512	8.033
Baseline Age	1.189	1.087	1.301
Baseline eGFR	0.973	0.953	0.993
Interaction Terms			
TKV:Age	0.982	0.972	0.993
eGFR:Age	0.998	0.998	0.999

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively

For each increase of 1 log TKV (i.e. TKV 1000 mls vs. 2738 mls), holding age and eGFR constant, there is an approximate 4.5-fold increase in the probability of ESRD. Baseline age and baseline eGFR were associated with hazard ratios of 1.189 and 0.973, respectively. These hazard ratios were derived assuming that other prognostic factors were kept constant, in addition to ignoring interaction terms.

5.3.4   ESRD - Exploratory Analyses Based on MRI/CT and US Modalities

Based on the request from the regulatory agencies to verify equivalence of the imaging modalities, each endpoint dataset was divided into two separate datasets (MRI/CT and US) to perform a Cox analysis. It is important to emphasize that the two modality datasets do not reflect a comparison of the same subjects using different modalities, but in fact are primarily different subject populations. Critical characteristics of these two subsets differ with regard to age and kidney function, where the MRI/CT subgroup is older and has lower kidney function or more progressive renal insufficiency. In addition, the MRI/CT and US modality datasets are smaller in sample size relative to the "all modality" dataset (i.e., a combination of all modalities).

Exploratory analyses were performed based on a dataset including TKV values measured with MRI and CT vs. US. This exploratory analysis was performed to examine potential differences in outcome

between the "MRI-CT" dataset relative to the "US" dataset. Similar analyses as described in Section 5.3.3 were performed. Summary results are presented in Appendix 8.8. Kaplan-Meier figures for the probability of ESRD for the MRI/CT and US datasets are presented in Figure 36.

Figure 36: Kaplan-Meier Plot for the Probability of ESRD Based on MRI/CT (Panel A) and US (Panel B) Modalities
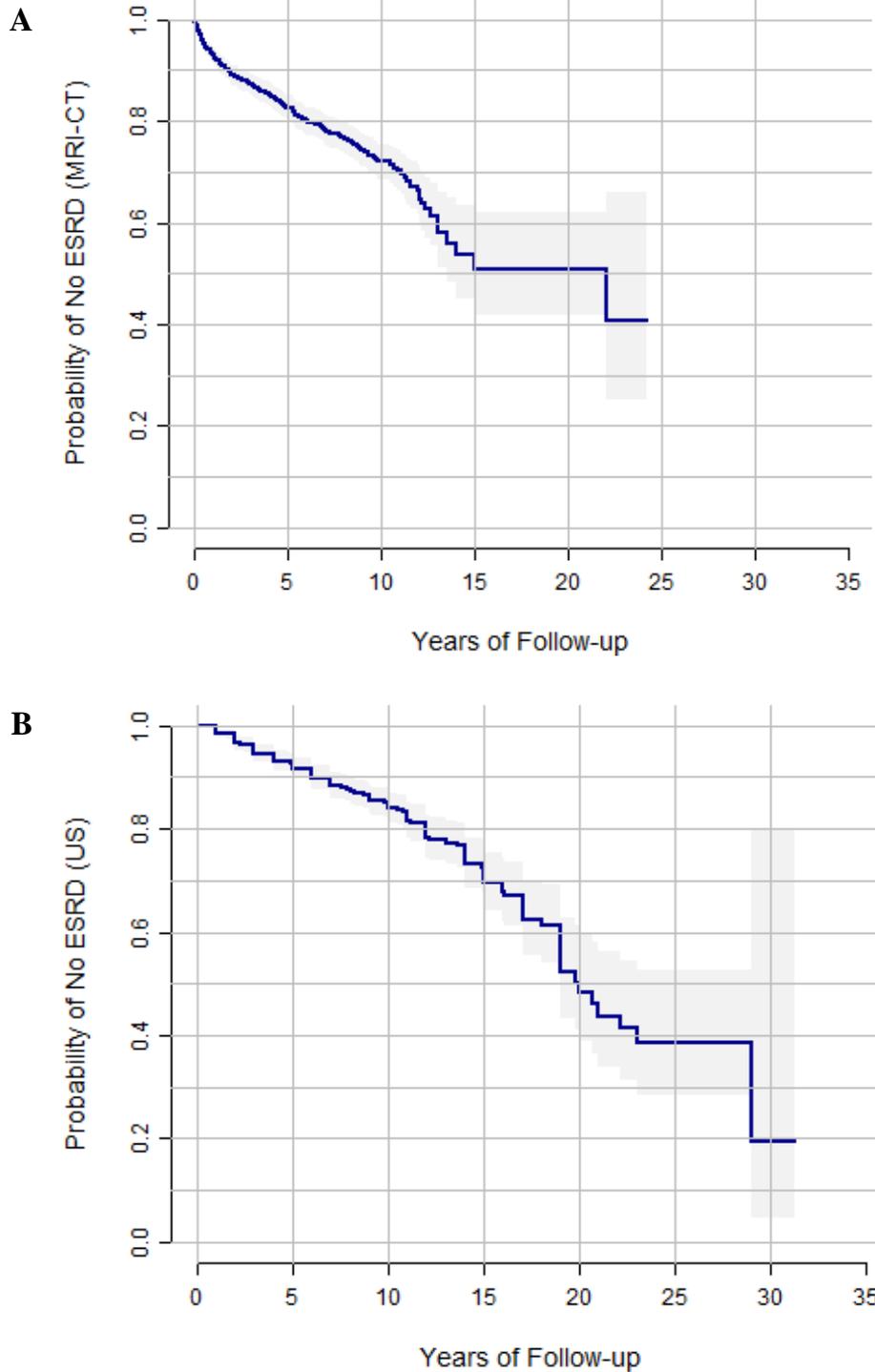
A decrease in the probability of no ESRD was observed relative to baseline age (median age = 40.04 years). The probability of no ESRD at 10 years for the MRI/CT and US datasets were approximately 72.5% and 85%, respectively (or conversely, a 27.5% and 15% increase in the probability of reaching ESRD at 10 years).

For the MRI/CT dataset, a total of 1561 patients with at least one TKV measurement were available in the database. A total of 445 patients with missing covariates were removed. The final dataset included 1116 patients of which with 235 (21.1%) developed ESRD. The final dataset did not have missing covariates, with the exception of two patients with missing height and 491 subjects with missing genotype information.

Parameters derived with the multivariate Cox model including interactions terms for the MRI/CT dataset are presented in Table 41.

Table 41: Multivariate Cox Model Including Interaction Terms for the Probability of ESRD – MRI/CT Modalities

Terms	coef	exp(Coefficient)	se(Coefficient)	z	P-value
Prognostic					
Baseline eGFR	-0.06598	0.936	0.017562	-3.757	0.00017
Ln Baseline TKV	0.72130	2.057	0.420877	1.714	0.08700
Baseline Age	0.05550	1.057	0.061220	0.907	0.36000
Interaction Terms					
eGFR:Age	-0.00100	0.999	0.000374	-2.679	0.00740
TKV:Age	-0.00571	0.994	0.007428	-0.769	0.44000

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively

Baseline eGFR was highly statistically significant (p=0.00017) while a statistical trend was observed for Ln Baseline TKV (p<0.1). Baseline age was not statistically significant. ROC values at one and five years were 0.8852 and 0.8307, respectively.

The above results are consistent with the more advanced disease stage of subjects in the MRI/CT dataset (mean age: 42.6 years, baseline TKV: 1.61 L and baseline eGFR: 64.3 mL/min) whereby baseline eGFR provided the most prognostic value (As opposed to the US dataset where the subjects had a less advanced disease stage and the corresponding baseline values were mean age: 31.7 years, baseline TKV: 1.15 L and baseline eGFR: 82.6 mL/min). Due to these differences in the baseline characteristics, and the smaller sample size of the MRI/CT dataset (relative to the all modality dataset), a multivariate Cox model without interaction terms was considered to avoid potential over-parameterization (see also Section 5.0).

Parameters derived with the multivariate Cox model without interaction terms for the MRI/CT dataset are presented in Table 42**.**

Table 42: Multivariate Cox Model without Interaction Terms for the Probability of ESRD – MRI/CT Modalities

Terms	coef	exp(Coefficient)	se(Coefficient)	z	P-value
Prognostic					
Baseline eGFR	-0.111	0.895	0.00617	-18.05	<0.00001
Ln Baseline TKV	0.404	1.498	0.10811	3.74	0.00019
Baseline Age	-0.016	0.984	0.00660	-2.42	0.01600

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively

The effect of baseline eGFR, Ln Baseline TKV and baseline age were all statistically significant. Based on the multivariate Cox model without interaction terms, ROC values at one and five years were 0.943 and 0.946, respectively.

For the US dataset, a total of 1140 patients with at least one TKV measurement were available in the database. A total of 363 patients with missing covariates were removed (of which 276 had missing baseline eGFR). The final analysis dataset included 804 patients, of which 150 (18.7%) presented ESRD. The final dataset did not have missing covariates, with the exception of four with missing height and 334 patients with missing genotype information. Parameters derived with multivariate Cox model including interactions terms for the US dataset are presented in Table 43.

Table 43: Multivariate Cox Model Including Interaction Terms for the Probability of ESRD – US Modality

Parameters	coef	exp(Coefficient)	se(Coefficient)	z	P-value
Prognostic					
Ln Baseline TKV	2.16831	8.744	0.526530	4.118	<0.0001
Baseline Age	0.28964	1.336	0.091666	3.160	0.0016
Baseline eGFR	-0.00786	0.992	0.014510	-0.541	0.590
Interaction Terms					
eGFR:Age	-0.00217	0.998	0.000375	-5.778	<0.0001
TKV:Age	-0.03106	0.969	0.011500	-2.701	0.0069

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively

Ln Baseline TKV was highly statistically significant (p<0.0001) and baseline age was statistically significant (i.e., p=0.0016). Baseline eGFR was not statistically significant. ROC values at one and five years were 0.8687and 0.8307, respectively. The above results are consistent with the less advanced disease stage of subjects in the US dataset (mean age: 31.7 years, baseline TKV: 1.15 L and baseline eGFR: 82.6 mL/min), (As opposed to the MRI/CT dataset where the subjects had a more advanced disease stage and the corresponding baseline values were mean age: 42.6 years, baseline TKV: 1.61 L and baseline eGFR: 64.3 mL/min.). Due to these differences in the baseline characteristics, and the smaller sample size in the US dataset (relative to the all modality dataset), a multivariate Cox model without interaction terms was considered to avoid potential over-parameterization (see also Section 5.0).

Parameters derived with the multivariate Cox model without interaction terms for the US dataset are presented in Table 44.

Table 44: Multivariate Cox Model without Interaction Terms for the Probability of ESRD – US Modality

Terms	coef	exp(Coefficient)	se(Coefficient)	z	P-value
Prognostic					
Ln Baseline TKV	0.6980	2.010	0.13787	5.06	<0.0001
Baseline Age	-0.0220	0.978	0.00992	-2.21	0.027
Baseline eGFR	-0.0902	0.914	0.00721	-12.51	<0.0001

Note: Hazard ratio for Ln baseline TKV, baseline age, and baseline eGFR were derived for a difference of 1 log (i.e., 2.7-fold increase in TKV), 1 year and 1 mL/min, respectively

The effect of baseline eGFR, Ln Baseline TKV and baseline age were all statistically significant. Based on the multivariate Cox model without interaction, ROC values at one and five years were 0.965 and 0.934, respectively.

Overall, statistical results derived with the MRI/CT and US datasets appeared to be similar when using multivariate Cox model without interaction. It is important to reemphasize that the two modality datasets do not reflect a comparison of the same subjects using different modalities but in fact are different groups of subjects. Results for the MRI/CT and US datasets should be interpreted with caution due to differences in mean age (42.6 vs. 31.7 years), baseline TKV (1.61 vs. 1.15 L) and baseline eGFR (64.3 vs.82.6 mL/min).

5.3.5   ESRD - Joint Model Buildup and Validation

Joint Model Buildup

A joint model linking the trajectory of TKV and the probability of ESRD was constructed as a potential drug development tool for trial enrichment. Simulations performed with the joint model may ultimately be used to select patient characteristics to predict disease progression in sub-populations of interest.

Multivariate Cox analyses have demonstrated that ln-transformed baseline TKV and baseline age were statistically significant predictors for the probability of ESRD based on a dataset including all imaging modalities (i.e., combined US, MRI and CT measurements). Furthermore, exploratory analyses performed with the multivariate Cox models demonstrated that results derived with the MRI/CT dataset were similar to those derived with the US dataset. Based on the above results, joint modeling of TKV and the probability of avoiding ESRD was performed using all imaging modalities.

A total of 866 patients with at least two TKV measurements separated by at least six months were included in the analysis and a total of 3258 TKV measurements were available across subjects. A total of 147 patients developed ESRD (17.0%). Descriptive statistics of baseline characteristics of patients included in the joint modeling analysis are provided in Appendix 8.8.

The following models were tested:

> **TKV Model**
> - A linear mixed-effect model with a random intercept (baseline ln-transformed TKV) was used to fit ln-transformed TKV over time
>
> **Event Model (Probability of ESRD)**

- Association parameter between predicted TKV at the time of ESRD was modeled using a Weibull model
- Baseline age, baseline eGFR, and interaction terms were found statistically significant.

Summary results of the joint modeling analysis are presented in Table 45.

Table 45: Final Parameters of the Joint Model for the Probability of ESRD

Parameters	Value	Standard Error	z-value	p-value
TKV Model				
Intercept	6.712	0.0301	223	<0.0001
Rate of Growth	0.056	0.002	28.4	<0.0001
Event Model				
Association (TKV→ Event)	1.2365	0.1631	7.581	<0.0001
Baseline eGFR	-0.0391	0.0135	-2.904	0.0037
Baseline Age	0.0355	0.0169	2.1	0.0357
eGFR:Age	-0.0012	0.0003	-3.646	0.0003

The rate of growth of TKV was 0.056 (corresponding to 5.60% per year). The association between the predicted TKV value at the time of ESRD (Weibull function) was highly statistically significant (p<0.0001). Baseline eGFR (p=0.0037) and baseline age (p=0.0357) were also statistically significant. Model adequacy was confirmed using pertinent graphics of goodness-of-fit for the longitudinal outcome (refer to Appendix 8.8).

Cross-Validation of Joint Model

Cross-validation was performed using a five-fold data-splitting method to evaluate the predictive performance of the final joint model. The predictive performance of the joint model was assessed by deriving mean prediction errors (MPE) and root mean square errors (RMSE) values for the probability of avoiding ESRD. Results of the cross-validation are presented in Table 46.

Table 46: Cross-Validation of Joint Model for the Predicted Probability of Avoiding ESRD

Parameters	Follow-Up Times	Predicted Error Median	Predicted Error Lower	Predicted Error Upper
Bias (MPE %)	1.00	0.23	0.68	-0.00
	3.00	1.62	3.29	0.03
	5.00	5.24	8.72	1.58
	10.00	13.50	16.80	7.37
Precision (RMSE %)	1.00	0.32	0.95	0.00
	3.00	1.08	2.11	0.15
	5.00	2.17	3.02	1.35
	10.00	3.77	3.17	2.94

Mean prediction errors (MPE) are computed as: (pred_val − obs_val)/obs_val * 100%, where obs_val and pred_val are the observed and predicted percentiles at the desired quantile (time) over the Test (or validation) group in the fold. Root mean square errors (RMSE) are computed as: $RMSE(\%) = \left[N^{-1} \cdot \sum_{i=1}^{N} (pe_i)^2 \right]^{1/2}$

Mean prediction errors (MPE) values for avoiding ESRD over 1, 3, 5 and 10 years of follow-up were 0.23%, 1.62%, 5.24% and 13.5%, respectively. Assuming a 75% probability of avoiding ESRD at 10 years of follow-up, the predicted error would be approximately 10% (i.e., 75% x 0.135).

Overall, the predicted probabilities of avoiding ESRD up to 10 years derived with the joint model were deemed acceptable.

The above results suggest that the joint model, along with TKV and other prognostic factor such as baseline age and baseline eGFR can accurately predict the risk of progression to ESRD over a prolonged follow-up period.

5.3.6   ESRD - Simulations

Simulations were performed with the final joint model to explore the effect of baseline TKV, baseline age and baseline eGFR. Simulation results in a typical 20-year-old subject according to baseline TKV values (500, 1000, 2000, and 3000 mL) and baseline eGFR (30, 50, and 70 mL/min/1.73m^2) over different follow-times (1, 3, 5, and 10 years) are presented in Table 47.

Table 47: Joint Model – Predicted Probability Avoiding ESRD in a Typical 20-year-old Subject as a Function of Baseline TKV and Baseline eGFR

Baseline Age	Baseline TKV (mL)	Follow-Up Times (Years)	Probability of No ESRD		
			eGFR 30 (mL/min/1.73m^2)	eGFR 50 (mL/min/1.73m^2)	eGFR 70 (mL/min/1.73m^2)
20	500	1	0.999	1.000	1.000
		3	**0.979**	0.994	**0.998**
		5	0.922	0.979	0.994
		10	0.569	0.865	0.953
	1000	1	0.997	0.999	1.000
		3	**0.955**	0.987	**0.996**
		5	0.846	0.954	0.987
		10	0.327	0.744	0.918
	2000	1	0.993	0.998	0.999
		3	**0.907**	0.973	**0.992**
		5	0.704	0.905	0.971
		10	0.123	0.551	0.842
	3000	1	0.989	0.997	0.999
		3	**0.858**	0.959	**0.987**
		5	0.583	0.861	0.955
		10	0.047	0.441	0.764

- Results for three years follow-up times in a typical 20-year-old subject are summarized below:
    - For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were

97.9%, 95.5%, 90.7% and 85.8%, respectively (or 2.1%, 4.5%, 9.3%, and 14.2% probability of <u>reaching</u> ESRD).

- o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 99.8%, 99.6%, 99.2% and 98.7%, respectively (or 0.2%, 0.4%, 0.8%, and 1.3% probability of <u>reaching</u> ESRD).

- Results for 10 years follow-up times in a typical 20-year-old subject are summarized below:

  - o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 56.9%, 32.7%, 12.3% and 4.7%, respectively (or 43.1%, 67.3%, 87.7%, and 95.3% probability of <u>reaching</u> ESRD).

  - o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 95.3%, 91.8%, 84.2% and 76.4%, respectively (or 4.7%, 8.2%, 15.8%, and 23.6% probability of <u>reaching</u> ESRD).

The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to progress to ESRD during a clinical trial.

Simulation results in a typical 40-year-old subject according to baseline TKV values (500, 1000, 2000, and 3000 mL) and baseline eGFR (30, 50, and 70 mL/min/1.73m^2) over different follow-times (1, 3, 5, and 10 years) are presented in Table 48.

Table 48: Joint Model –Predicted Probability Avoiding ESRD in a Typical 40-year-old Subject as a Function of Baseline TKV and Baseline eGFR

Baseline Age	Baseline TKV (mL)	Follow-Up Times (Years)	Probability of No ESRD		
			eGFR 30 (mL/min/1.73m^2)	eGFR 50 (mL/min/1.73m^2)	eGFR 70 (mL/min/1.73m^2)
40	500	1	0.999	1.000	1.000
		3	**0.980**	0.996	**0.999**
		5	0.923	0.986	0.998
		10	0.581	0.912	0.982
	1000	1	0.997	0.999	1.000
		3	**0.957**	0.992	**0.999**
		5	0.853	0.971	0.995
		10	0.338	0.822	0.966
	2000	1	0.993	0.999	1.000
		3	**0.908**	0.984	**0.997**
		5	0.711	0.944	0.989
		10	0.142	0.702	0.937
	3000	1	0.989	0.998	1.000
		3	**0.860**	0.974	**0.995**
		5	0.593	0.913	0.984
		10	0.050	0.584	0.910

- Results for three years follow-up times in a typical 40-year-old subject are summarized below:

    o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 98.0%, 95.7%, 90.8% and 86.0%, respectively (or 2.0%, 4.3%, 9.2%, and 14.0% probability of <u>reaching</u> ESRD).

    o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 99.9%, 99.9%, 99.7% and 99.5%, respectively (or 0.1%, 0.1%, 0.3%, and 0.5% probability of <u>reaching</u> ESRD).

- Results for 10 years follow-up times in a typical 40-year-old subject are summarized below:

    o For a typical baseline eGFR of 30 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 58.1%, 33.8%, 14.2% and 5.0%, respectively (or 41.9%, 66.2%, 85.8%, and 95.0% probability of <u>reaching</u> ESRD).

    o For a typical baseline eGFR of 70 mL/min/1.73m^2, mean predicted probabilities of avoiding ESRD for baseline TKV values of 500, 1000, 2000 and 3000 mL were 98.2%, 96.6%, 93.7% and 91.0%, respectively (or 1.8%, 3.4%, 6.3%, and 9.0% probability of <u>reaching</u> ESRD).

The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to progress to ESRD during a clinical trial. A randomized control trial (RCT) with ESRD as an endpoint in those with relatively intact kidney function would need extremely large numbers of subjects and an extremely long time enrolled in the RCT, making feasibility unlikely unless the patient population had a very large TKV.

6   Summary

The goal of this project is to qualify TKV as a prognostic biomarker for clinical trial enrichment with patients with ADPKD. Analyses were performed to determine whether sufficient evidence exists to qualify TKV in combination with patient age and baseline eGFR, to accurately predict the risk and cadence of disease progression in ADPKD patients. TKV measurements were performed using CT, MRI, and ultrasound (US) in the database, which included patients from three registries (University of Colorado – Denver, Emory University, and Mayo Clinic) and CRISP 1 and 2 prospective cohort studies.

The following disease outcomes for ADPKD patients were considered in the analysis:

- 30% Worsening of eGFR
- 57% Worsening of eGFR
- ESRD

After analyzing the differences between the subsets of data based on modality, the current analysis included the data from all imaging modalities.

6.1 30% Worsening of eGFR

Based on the PKDOC dataset, a 30% decline in eGFR relative to baseline was used to derive the endpoint. A subsequent eGFR measurement was required to confirm the original decline ("restrictive" definition of the endpoint). TKV values measured by MRI, CT, or US modalities were used in the analysis (referred to as the "All Modalities" dataset).

A multivariate Cox model was developed to identify prognostic factors for a 30% worsening of eGFR in ADPKD patients. A total of 2355 patients with at least one TKV measurement in the database were available. After removing patients with missing covariates, the dataset included 1140 patients of which 361 (31.7%) presented a 30% worsening of eGFR.

- The final multivariate Cox model included ln-transformed baseline TKV, baseline age, baseline eGFR, and the following interaction terms: 1) baseline eGFR and baseline age, 2) ln-transformed baseline TKV and baseline age, and 3) baseline eGFR and ln-transformed baseline TKV. Based on ROC values (predictive value of model), as well as Z and p-values (statistical contribution of individual prognostic factors), these results suggest that ln-transformed baseline TKV is the most important prognostic biomarker of progression to 30% worsening of eGFR, though age and eGFR also contribute independent predictive information.

- Ln-transformed baseline TKV was associated with a hazard ratio (95% CI) of 15. 723 (7.652 - 32.303). Baseline age and baseline eGFR were associated with hazard ratios of 1.297 and 1.01, respectively. These hazard ratios should be interpreted with caution since they were derived assuming that other prognostic factors were kept constant in addition to ignoring interaction terms.

- An exploratory analysis was performed to examine potential differences in outcome between the "MRI-CT" dataset relative to the "US" dataset. Similar results were observed in the MRI-CT dataset and US dataset.

A formal analysis was performed by simultaneously modeling longitudinal TKV values and the probability of avoiding a 30% worsening of eGFR with a joint model. A total of 641 patients with at least two TKV measurements separated by at least six months were included in the analysis. A total of 192 patients presented a 30% worsening of eGFR (30.0%).

- The rate of growth of TKV was 0.0516 (corresponding to 5.16% per year). The association between the predicted TKV at the time of 30% worsening of eGFR (piecewise linear hazard function) was highly statistically significant (p<0.0001).

- The predictive performance of the joint model was assessed using a cross-validation methodology. The predicted probabilities of no 30% worsening of eGFR over 10 years derived with the joint model and the prognostic factors in ADPKD patients were within 2% of observed probabilities. The above results suggest that the joint model, along with TKV and other prognostic factor such as baseline age and baseline eGFR can accurately predict the risk of 30% worsening of eGFR over a prolonged follow-up period.

- The final joint model was used to simulate probabilities of a 30% worsening of eGFR as a function of baseline TKV, baseline eGFR and baseline age.

6.2   57% Worsening of eGFR

Based on the PKDOC dataset, a 57% decline in eGFR relative to baseline was used to derive the endpoint. A subsequent eGFR measurement was required to confirm the original decline ("restrictive" definition of the endpoint). TKV values measured by MRI, CT, or US modalities were used in the analysis (referred to as the "All Modalities" dataset).

A multivariate Cox model was developed to identify prognostic factors for a 57% worsening of eGFR in ADPKD Patients. A total of 2355 patients with at least one TKV measurement in the database were available. After removing patients with missing covariates, the analysis dataset included 1140 patients of which 115 (10.1%) presented a 57% worsening of eGFR.

- The final multivariate Cox model included statistically significant effects of ln-transformed baseline TKV (p<0.0001) and baseline age (p=0.014). The multivariate Cox model was customized by including the following interaction terms: 1) baseline eGFR and baseline age, and 2) ln-transformed baseline TKV and baseline age. Based on ROC values (predictive value of model), as well as Z and p-values (statistical contribution of individual prognostic factors), these results suggest that ln-transformed baseline TKV was the most important prognostic biomarker of disease progression in ADPKD patients. The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to show a 57% worsening of eGFR during a clinical trial.

- Ln-transformed baseline TKV was associated with a hazard ratio (95% CI) of 10.149 (3.432 – 30.012). Baseline age and baseline eGFR were associated with hazard ratios of 1.265 and 1.005, respectively. These hazard ratios should be interpreted with caution since they were derived assuming that other prognostic factors were kept constant in addition to ignoring interaction terms.

- An exploratory analysis was performed to examine potential differences in outcome between the "MRI-CT" dataset relative to the "US" dataset. Similar results were observed in the MRI-CT dataset and US dataset.

A formal analysis was performed by simultaneously modeling longitudinal TKV values and the probability of avoiding a 57% worsening of eGFR with a joint model. A total of 703 patients with at least two TKV measurements separated by at least six months were included in the analysis. A total of 59 patients presented a 57% worsening of eGFR (8.4%).

- The rate of growth of TKV was 0.0538 (corresponding to 5.38% growth per year). The association between the predicted TKV at the time of 57% worsening of eGFR (piecewise linear hazard function) was highly statistically significant (p<0.0001). Baseline eGFR was statistically significant (p=0.0009). The effect of baseline age was not statistically significant (p=0.8704).

- The predictive performance of the joint model was assessed using a cross-validation methodology. The predicted probabilities of avoiding a 57% worsening of eGFR over 10

years derived with the joint model and the prognostic factors in ADPKD patients were approximately within 5% of observed probabilities. The above results suggest that the joint model, along with TKV and other prognostic factor can accurately predict the risk of 57% worsening of eGFR over a prolonged follow-up period.

- The final joint model was used to simulate probabilities of a 57% worsening of eGFR as a function of baseline TKV, baseline eGFR and baseline age.

6.3   ESRD

Information on ESRD was specifically provided in the PKDOC database. TKV values measured by MRI, CT, or US modalities were used (referred to as the "All Modalities" dataset).

A multivariate Cox model was developed to identify prognostic factors for a progression to ESRD in ADPKD patients. A total of 2355 patients with at least one TKV measurement in the database were available. After removing patients with missing covariates, the analysis dataset included 1619 patients of which 354 (21.9%) presented ESRD.

- The final multivariate Cox model included statistically significant effects of ln-transformed baseline TKV (p<0.0001), baseline age (p=0.00016) and baseline eGFR (p=0.0095). The multivariate Cox model was customized by including the following interaction terms: 1) ln-transformed baseline TKV and baseline age, and 2) baseline eGFR and baseline age. Based on ROC values (predictive value of model), as well as Z and p-values (statistical contribution of individual prognostic factors), these suggest that ln-transformed baseline TKV was the most important prognostic biomarker of disease progression in ADPKD patients. The above information on baseline TKV can be used as inclusion criterion in clinical trials to identify patients likely to progress to ESRD during a clinical trial.

- Ln-transformed baseline TKV was associated with a hazard ratio (95% CI) of 4.492 (2.512 – 8.033) for the probability of ESRD. Baseline age and baseline eGFR were associated with hazard ratios of 1.189 and 0.973, respectively. These hazard ratios should be interpreted with caution since they were derived assuming that other prognostic factors were kept constant in addition to ignoring interaction terms.

- An exploratory analysis was performed to examine potential differences in outcome between the "MRI-CT" dataset relative to the "US" dataset. Similar results were observed in the MRI-CT dataset and US dataset.

A formal analysis was performed by simultaneously modeling longitudinal TKV values and the probability of avoiding ESRD with a joint model. A total of 866 patients with at least two TKV measurements separated by at least six months were included in the analysis. A total of 147 patients presented ESRD (17.0%).

- The rate of growth of TKV was 0.056 (corresponding to 5.60% per year). The association between the predicted TKV at the time of 57% worsening of eGFR (piecewise linear hazard function) was highly statistically significant (p<0.0001). Baseline eGFR (p=0.0037) and baseline age (p=0.0357) were statistically significant.

- The predictive performance of the joint model was assessed using a cross-validation methodology. The predicted probabilities of avoiding ESRD over 10 years derived with the joint model and the prognostic factors in ADPKD patients were within 10% of observed probabilities. Assuming a 75% probability of avoiding ESRD at 10 years of follow-up, the predicted error would be approximately 10% (i.e., 75% x 0. 135). The above results suggest that the joint model, along with TKV and other prognostic factors can accurately predict the risk of ESRD over a prolonged follow-up period.

- The final joint model was used to simulate probabilities of avoiding ESRD as a function of baseline TKV, baseline eGFR and baseline age.

6.4   Relative Importance of Biomarker Covariates

The relative importance of baseline TKV was evaluated in sub-groups of baseline age and baseline eGFR. The predicted probabilities of a 30% worsening of eGFR according to baseline TKV are presented in Figure 37.

Figure 37: Trial Enrichment Example - Mean (95%) Predicted Probabilities of a 30% Worsening of eGFR and Relative Effect of Baseline TKV
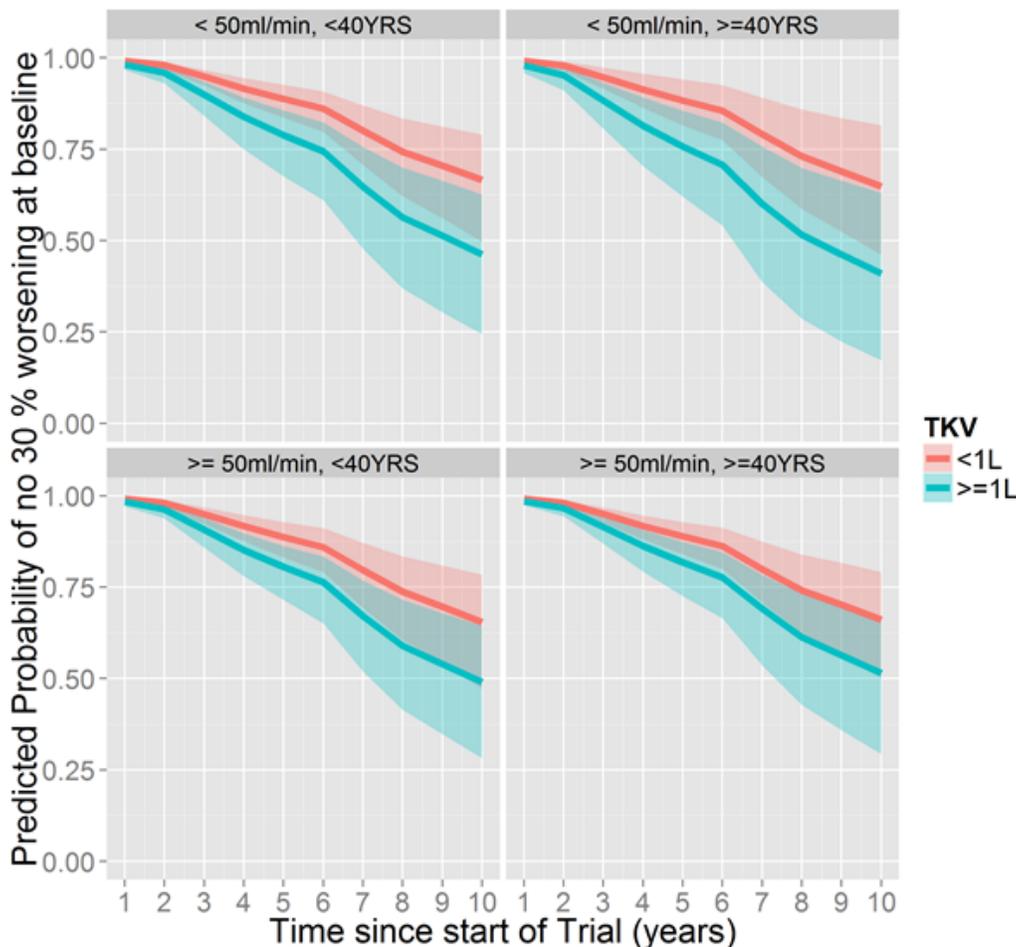
Patients with larger TKV (≥ 1 L) displayed steeper slopes of hazard functions, which translated into a higher risk of a 30% worsening of eGFR within each baseline age (< or ≥ 40 years) or baseline eGFR (< or ≥ 50 mL/min/1.73m^2) subgroups. These results suggest that patients with larger TKV are more likely to progress to a 30% worsening of eGFR, independent of other baseline characteristics.

The relative importance of baseline age was evaluated in sub-groups of baseline eGFR and baseline TKV. The predicted probabilities of a 30% worsening of eGFR according to baseline age are presented in Figure 38.

Figure 38: Trial Enrichment Example - Mean (95%) Predicted Probabilities of a 30% Worsening of eGFR and Relative Effect of Baseline Age
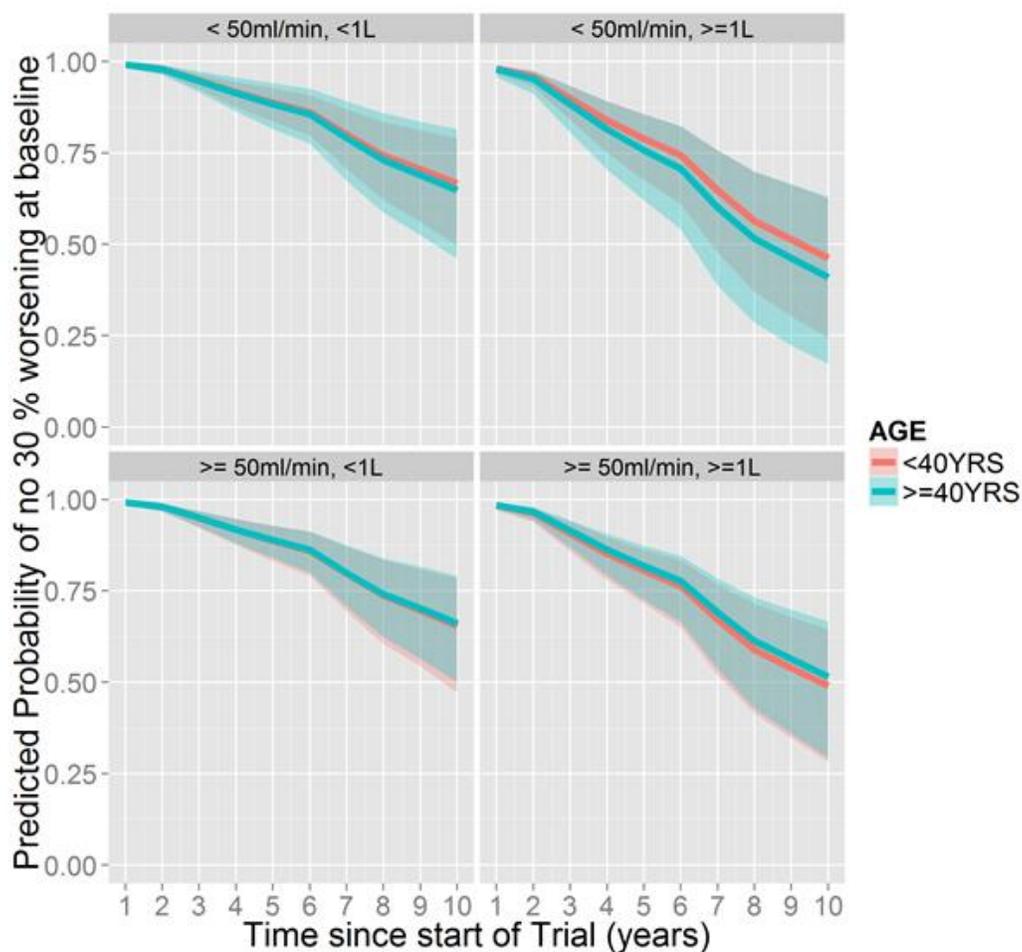
Predicted probabilities of 30% worsening of eGFR in patients < 40 years were similar to those in patients ≥ 40 years of age.

The relative importance of baseline eGFR was evaluated in sub-groups of baseline age and baseline TKV. The predicted probabilities of a 30% worsening of eGFR according to baseline eGFR are presented in Figure 39.

Figure 39: Trial Enrichment Example - Mean (95%) Predicted Probabilities of a 30% Worsening of eGFR and Relative Effect of Baseline aGFR
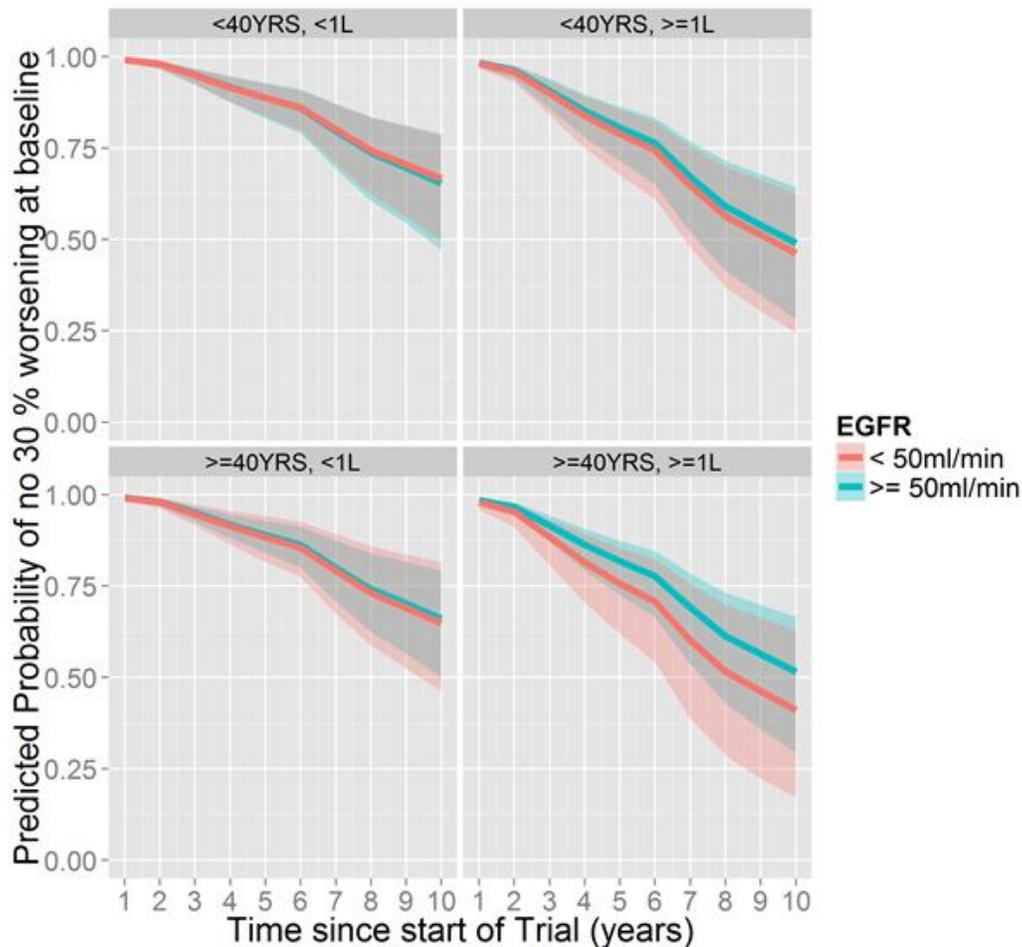
Predicted probabilities of 30% worsening of eGFR in patients with baseline eGFR < 50 mL/min/1.73m^2 years were similar to those in patients with baseline eGFR ≥ 50 mL/min/1.73m^2 in all sub-groups, with the exception of patients ≥ 40 years of age and baseline TKV ≥ 1 L (lower right panel).

6.5   Decision Tree for Use of Baseline TKV and Age for Prognostic Clinical Trial Enrichment

Overall, the current analysis suggests that ln-transformed baseline TKV can be applied as a prognostic biomarker that, in combination with baseline eGFR and baseline age, can accurately predict the risk and cadence of disease progression in ADPKD patients.

The above results may be used as a basis for trial enrichment, a concept in clinical trial design that attempts to improve the likelihood of clinical trial success by identifying a patient population that can

discriminate between active and inactive drug treatment. More precisely, statistical calculations may be performed to determine the sample size for specific clinical cut-offs, patient characteristics, and study duration that would provide sufficient power to detect statistically and clinically relevant differences between candidate drugs vs. placebo. For example the following decision tree may be considered to design optimal trials to 1) prevent early disease outcome, 2) reduce complications, or 3) reduce the progression to ESRD according to different disease endpoints.

Figure 40 illustrates how TKV, in combination with baseline eGFR and baseline age can be used in patient selection for clinical trials, and the resulting benefits.

Figure 40: Decision Tree for Use of Baseline TKV and Age for Prognostic Clinical Trial Enrichment
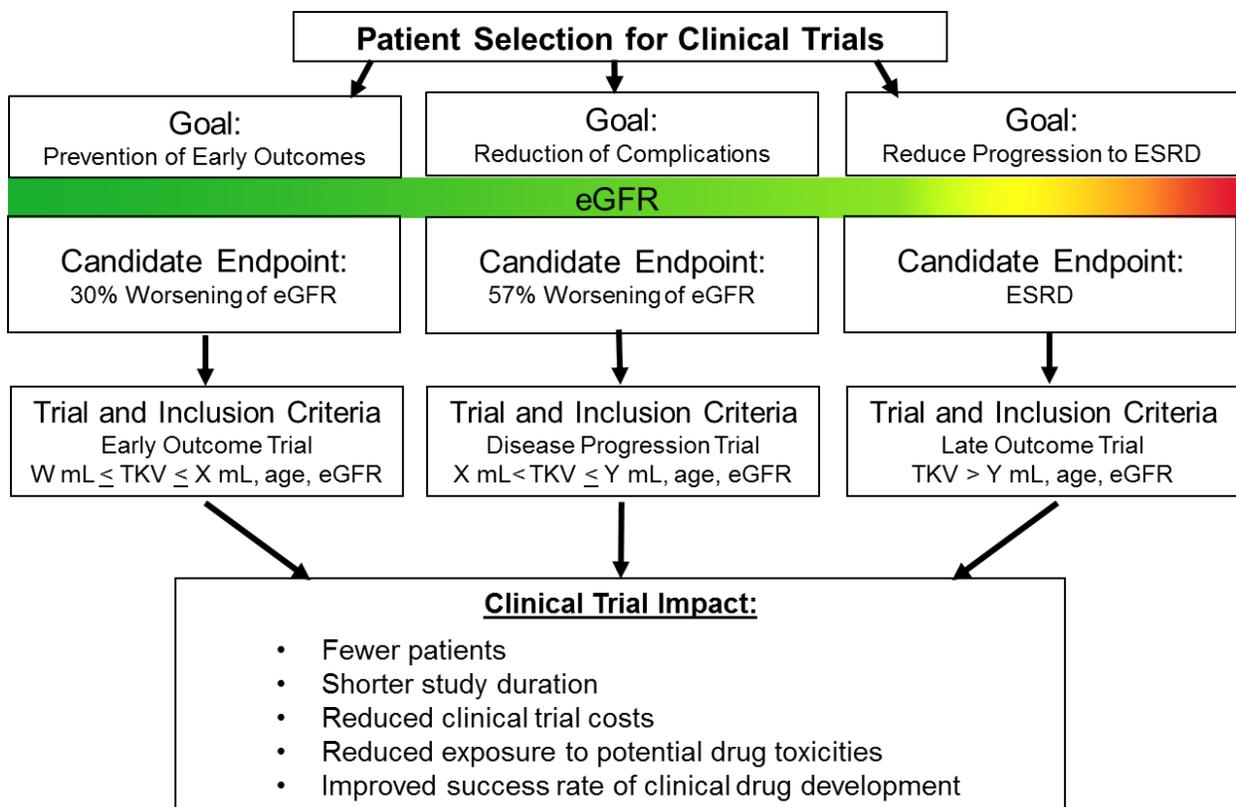
In order to explore potential scenarios of trial enrichment, the final joint model was used to simulate probabilities of a 30% worsening of eGFR as a function of pre-specified cut-offs of baseline TKV, baseline eGFR, and baseline age. Predicted probabilities of a 30% Worsening of eGFR according to baseline TKV (< 1 or ≥ 1 L), baseline age (< 40 and ≥40 years) and baseline eGFR (≥ 50 or < 50 mL/min/1.73m^2) over time are presented in Table 49.

Table 49: Trial Enrichment Example - Tabular Presentation of Predicted Probabilities of a 30% Worsening of eGFR According to Pre-Specified Baseline Age, Baseline TKV and Baseline eGFR Cut-Offs

Follow-Up Times (Years)	Probabilities of Avoiding 30% Worsening of eGFR							
	TKV < 1 L				TKV ≥ 1 L			
	Age: < 40 years		Age: ≥ 40 years		Age: < 40 years		Age: ≥ 40 years	
	eGFR ≥ 50 mL/min	eGFR < 50 mL/min	eGFR ≥ 50 mL/min	eGFR < 50 mL/min	eGFR ≥ 50 mL/min	eGFR < 50 mL/min	eGFR ≥ 50 mL/min	eGFR < 50 mL/min
1	0.991	0.992	0.992	0.991	0.984	0.982	0.985	0.979
2	0.980	0.980	0.981	0.979	0.963	0.959	0.966	0.953
3	0.950	0.949	0.951	0.947	0.907	**0.899**	0.915	**0.884**
4	0.917	0.916	0.918	0.913	0.852	0.839	0.863	0.815
5	0.887	0.888	0.889	0.884	**0.805**	0.789	0.818	**0.757**

For example, the following trial may be considered:

- A trial performed over three years may be considered for reaching a 30% Worsening of eGFR in patients with baseline TKV ≥ 1 L and

  o   < 40 years and baseline eGFR < 50 mL/min/1.73m^2 (i.e., probability of 10.1%)

  o   ≥ 40 years and baseline eGFR < 50 mL/min/1.73m^2 (probability of 12.6%)

- If a trial in younger patients (age < 40 years) with preserved renal function (eGFR >50 mL/min/1.73m2) is desired, a larger baseline TKV (TKV >1 L) would increase the probability of reaching the 30% reduction in 5 years to approximately 20%.

- A disease progression trial performed over five years may be considered for reaching a 30% Worsening of eGFR in patients with TKV ≥ 1 L, regardless of baseline age and baseline eGFR (range of probabilities: 19.5 – 24.3%).

- The above trial enrichment scenarios suggest that baseline TKV is the most important prognostic biomarker of disease progression in ADPKD patients.

Based on the above probabilities, statistical power calculations may be performed to determine the sample size (amount/percent of enrichment) for the above disease endpoints, according to patient characteristics (age, baseline eGFR, and baseline TKV and respective cut-off points for inclusion/exclusion criteria for enrollment) and study duration (follow-up times) that would provide sufficient power to detect statistically and clinically relevant differences between a candidate drug with a hypothetical effect on TKV and the resulting effect on the probabilities of disease outcome vs. a control arm (e.g., placebo treatment).

Sponsors may perform additional analysis to determine an optimal study design (study duration, sample size, and patient characteristics) that will optimize statistical power. Pharmaceutical sponsors and academic investigators will be encouraged to prospectively employ the above joint model in designing future trials similar to others models that have been made publicly available by FDA.

6.6   Conclusions

In conclusion, the PKDOC has provided an overview of the significant unmet need for effective medications to treat ADPKD. Tremendous scientific progress has been made in understanding the underlying mechanisms of disease and pathophysiological processes underlying ADPKD. This has resulted in several potential drug therapy targets, some of which have shown great promise in animal studies. However, most sponsors have been reluctant to invest in drug development because of the lack of clear, viable regulatory endpoints, specifically because the current traditional endpoints measuring renal function only show changes when the disease is very advanced and hold little chance for any treatment effect. The PKDOC was created as a collaborative call to action by the world's leading scientific and clinical experts in ADPKD together with the voice of the patient, through the Polycystic Kidney Disease Foundation, and the regulatory expertise of the Critical Path Institute.

The PKDOC has identified TKV as an imaging biomarker that is most relevant for tracking and predicting the natural history of ADPKD. There is evidence in the literature from both animal and human studies to support TKV as a prognostic biomarker for use in clinical trials for ADPKD. However, the data previously available are in the form of anecdotal reports, or clinical studies with small number of patients and for limited periods of time. In discussions with the FDA, PKDOC has developed the first-ever CDISC data standards for ADPKD to allow for the mapping and pooling of available data into a common dataset that has been used to support the regulatory qualification of TKV by the FDA and EMA. This common dataset is one of the largest ever datasets of ADPKD patients, with a total of 2355 patients. The PKDOC dataset includes 1182 subjects who have at least two images for the measurement of TKV taken at least six months apart. This rich and robust dataset has allowed the PKDOC to develop a disease analysis model that links baseline TKV, baseline eGFR, and baseline age with clinical outcomes of ADPKD, supporting the regulatory qualification of this biomarker.

Conclusion: The above results confirm that ln-transformed baseline TKV was the most significant prognostic biomarker of disease progression in ADPKD patients. Baseline TKV can be used as a prognostic biomarker to identify patients likely to show a (1) 30% worsening of eGFR, (2) a 57% worsening of eGFR, or (3) ESRD during a clinical trial. Patient age and baseline eGFR are significant covariates that enrich the prognostic ability of TKV in selecting patients for clinical trials.

This outcome of this qualification effort is eagerly anticipated by patients suffering from ADPKD and their families, all of whom are anxious to see an effective treatment option. The PKDOC is very grateful to the FDA and EMA for the support, encouragement, and guidance they have received, and continue to receive. We are proud to be collaborating with the FDA and EMA on this critically important project to bring hope to, and ease suffering of patients and their families with ADPKD.

6.7   Future Plans

It is the desire and intent of the clinicians and pharmaceutical companies participating in PKDOC to eventually qualify TKV as a new primary endpoint in clinical trials. Currently, sponsors are reluctant to invest in drug development of potentially promising compounds in the absence of a clear and viable regulatory path for clinical trial design. Accepted regulatory endpoints for clinical trials designed to slow progression of chronic kidney disease are presently limited to development of kidney failure requiring renal replacement therapy and doubling of serum creatinine (sCr) {Levey 2009}. Because progression of ADPKD occurs over many decades, use of such endpoints would require studies to focus on late-stage disease, a stage when patients are not likely to respond to an intervention. Alternatively, clinical trials would need to be decades in duration, clearly something which potential sponsors are not likely to pursue. When using subjects likely to benefit from therapy that could slow disease progression, the requirement to reach kidney failure as an outcome means that the time frame for performing a clinical trial (even with an earlier intervention) could be a decade or more, beyond the resources of any federal or private entity.

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