ELSEVIER

CrossMark

Urologic Oncology: Seminars and Original Investigations 35 (2017) 529.e9–529.e16

UROLOGIC ONCOLOGY

Original article

Description of the EuroTARGET cohort: A European collaborative project on TArgeted therapy in renal cell cancer—GEnetic- and tumor-related biomarkers for response and toxicity

Loes F.M. van der Zanden, Ph.D.[a,1], Sita H. Vermeulen, Ph.D.[a,1], Arna Oskarsdottir, M.Sc.[b], Jake S.F. Maurits, M.Sc.[a], Meta H.M. Diekstra, M.Pharm.[c], Valentin Ambert, M.D.[d], Anne Cambon-Thomsen, M.D., D.R., C.N.R.S.[e], Daniel Castellano[f], Achim Fritsch, R.Ph.[g], Jesus Garcia Donas, M.D., Ph.D.[h], Rosa Guarch Troyas, M.D.[i], Henk-Jan Guchelaar, Pharm.D., Ph.D.[c], Arndt Hartmann, M.D.[j], Christina Hulsbergen-van de Kaa, M.D., Ph.D.[k], Ulrich Jaehde, Ph.D.[g], Kerstin Junker, Ph.D.[l], Anna Martinez-Cardus, Ph.D.[m], Gisli Masson, Ph.D.[b], Jeannette Oosterwijk-Wakka, Ph.D.[n], Marius T. Radu, M.D.[d], Thorunn Rafnar, Ph.D.[b], Cristina Rodriguez-Antona, Ph.D.[o], Max Roessler, Ph.D.[p], Rob Ruijtenbeek, Ph.D.[q], Kari Stefansson, M.D., Ph.D.[b,r], Anne Warren, M.B.B.S., M.Sc., F.R.C.Path.[s], Lodewyk Wessels, Ph.D.[t], Tim Eisen, F.R.C.P., Ph.D.[u], Lambertus A.L.M. Kiemeney, Ph.D.[a,*,2], Egbert Oosterwijk, Ph.D.[n,2]

[a] *Radboud University Medical Center, Radboud Institute for Health Sciences, Nijmegen, The Netherlands*
[b] *deCODE Genetics/Amgen, Reykjavik, Iceland*
[c] *Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands*
[d] *University of Medicine and Pharmacy Carol Davila, Bucaresti, Romania, Bucuresti, Romania*
[e] *Epidemiology and analyses in public health, Joint Unit 1027, Institut National de la Santé et de la Recherche Médicale (INSERM), Université Toulouse III Paul Sabatier, Faculty of Medicine, Toulouse, France*
[f] *Medical Oncology Department, Hospital Universitario 12 de Octubre, I + 12 Research Institute, (CiberOnc), Madrid, Spain*
[g] *Institute of Pharmacy, Clinical Pharmacy, University of Bonn, Bonn, Germany*
[h] *Medical Oncology, HM Hospitales—Centro Integral Oncológico HM Clara Campal, Madrid, Spain*
[i] *Anatomía Patológica, Complejo Hospitalario de Navarra, Pamplona, Spain*
[j] *Department of Pathology, University Erlangen-Nürnberg, Erlangen, Germany*
[k] *Department of Pathology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands*
[l] *Clinic of Urology and Paediatric Urology, Saarland University, Homburg, Germany*
[m] *Cancer Epigenetics and Biology Program, Bellvitge Biomedical Research Institute, Barcelona, Catalonia, Spain*
[n] *Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands*
[o] *Hereditary Endorine Cancer Group, Spanish National Cancer Research Centre (CNIO) and Biomedical Network on Rare Diseases (CIBERER), Madrid, Spain*
[p] *CESAR central office, CESAR Central European Society for Anticancer Drug Research-EWIV, Vienna, Austria*
[q] *PamGene International B.V., 's-Hertogenbosch, The Netherlands*
[r] *Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavík, Iceland*
[s] *Department of Histopathology, Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge, UK*
[t] *Department of Molecular Carcinogenesis, The Netherlands Cancer Institute, Amsterdam, The Netherlands*
[u] *Department of Oncology, Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge, UK*

Received 20 February 2017; accepted 4 March 2017

Funding sources: EuroTARGET has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under Grant agreement no. 259939.

[1]Shared first authorship.

[2]Shared last authorship.

[*] Corresponding author. Tel.: +31-24-361-3745.
E-mail address: Bart.Kiemeney@radboudumc.nl (Lambertus A.L.M. Kiemeney).

http://dx.doi.org/10.1016/j.urolonc.2017.03.009

Abstract

Objective: For patients with metastatic renal cell cancer (mRCC), treatment choice is mainly based on clinical parameters. With many treatments available and the limited response to treatment and associated toxicities, there is much interest in identifying better biomarkers for personalized treatment. EuroTARGET aims to identify and characterize host- and tumor-related biomarkers for prediction of response to tyrosine kinase inhibitor therapy in mRCC. Here, we describe the EuroTARGET mRCC patient cohort.

Methods and materials: EuroTARGET is a European collaborative project designed as an observational study for which patients with mRCC were recruited prospectively in 62 centers. In addition, 462 patients with mRCC from previous studies were included. Detailed clinical information (baseline and follow-up) from all patients was entered in web-based case record forms. Blood was collected for germline DNA and pharmacokinetic/pharmacodynamic analyses and, where available, fresh-frozen tumor material was collected to perform tumor DNA, RNA, kinome, and methylome analyses.

Results: In total, 1,210 patients with mRCC were included. Of these, 920 received a tyrosine kinase inhibitor as first-line targeted treatment (sunitinib [$N = 713$, 78%], sorafenib [$N = 41$, 4%], or pazopanib [$N = 166$, 18%]) and had at least 6 months of outcome assessment (median follow-up 15.3 months [interquartile range: 8.5–30.2 months]). Germline DNA samples were available from 824 of these patients, fresh-frozen tumor material from 142 patients, fresh-frozen normal kidney tissue from 95 patients, and tissue microarrays created from formalin-fixed paraffin-embedded tumor material from 247 patients. Of the 920 patients, germline DNA variant chip data were successfully generated for 811 patients (Illumina HumanOmniExpress BeadChip). For 80 patients, next-generation exome sequencing of germline and tumor DNA was performed, tumor RNA sequencing was performed for 124 patients, kinome activity measured and processed for 121 patients (PamChip), and methylome data (Illumina Infinium HumanMethylation450 BeadChip) were created for 116 RCC tissues (and 23 normal kidney tissues). For 73 out of the 920 patients, all platform data types were generated. In addition, 40 patients were included in a pharmacokinetic/pharmacodynamic phase IV substudy.

Conclusions: Analysis of EuroTARGET cohort data will contribute to personalization of therapy for patients with mRCC. The extensive clinical data and multiplatform EuroTARGET data will be freely available.

Keywords: Metastatic renal cell carcinoma; Therapy response; Tyrosine kinase inhibitor; Biomarker; Transcriptomics; Genomics

1. Introduction

With more than 121,000 newly diagnosed patients and 52,000 deaths each year, kidney cancer is the seventh most common cancer in Europe [1]. Further, 90% of all kidney cancers are renal-cell carcinomas (RCC). The prognosis of RCC is highly dependent on stage. Surgery is effective for the 70% to 80% of patients with localized disease, leading to 5-year relative survival rates of more than 70% [2]. However, ~25% of patients have metastatic RCC (mRCC) at first diagnosis, and ~25% of patients with localized disease develop metastases after surgery [3,4].

Until the arrival of tyrosine kinase inhibitors (TKIs), treatment options in mRCC were limited, and 5-year relative survival was only 5% to 10%. Randomized clinical trials showed that TKI agents, directly targeting tumorigenic and angiogenic pathways (reviewed in [5]), significantly improved the outcomes of these patients [6]. Several first-line TKI treatment options are now available, such as sunitinib, pazopanib, or bevacizumab plus interferon-alpha [6]. Most patients experience disease stabilization or response for a median of ~12 months [7]. However, TKI treatment is extremely expensive (sunitinib was estimated in the UK to cost £71,462 per quality adjusted life year gained [8]), 15% to 20% of patients experience immediate disease progression despite treatment [7], nearly all patients eventually become resistant, and toxicity is common and leads to dose reduction. In addition, first-line treatment options for patients with mRCC will likely increase in coming years (including immune checkpoint inhibition) [9]. There is, hence, much interest in tools for prediction of individual therapy response and acquired resistance to TKIs to optimize treatment outcome while reducing unnecessary drug use and expenses, and improving human health and quality of life.

Currently, treatment choice in mRCC is based on risk grouping of patients by clinical parameters such as the patient's performance status, and serum biochemical measurements, and histological features of the tumor [10]. A comparison study into several clinical risk grouping models, including that of the International Metastastic Renal Cell Carcinoma Database Consortium [11] and the Memorial Sloan Kettering Cancer Center (MSKCC) [12], showed modest discriminatory values for survival (area under the receiver operating curve ~0.66) and indicated that addition of tumor-specific or patient-specific biomarkers is likely required for the improvement of the accuracy of these models [13].

Advances in high-throughput technologies have paved the way to personalized medicine using biomarkers. For mRCC, potential prognostic molecular biomarkers such as *PBRM1*, *BAP1*, and *KDM5C* tumor mutations [14]; IL8 [15], VEGF, and PlGF levels [16]; *ABCB1* and *VEGFR-3* germline polymorphisms [17]; and miRNA levels have been identified [18,19]. However, there are no validated biomarkers yet that can guide personalization of therapy in patients with mRCC.

In this framework EuroTARGET was initiated, a "European collaborative project on TArgeted therapy in Renal cell cancer: GEnetic and Tumor-related biomarkers for response and toxicity." The overarching goal of this

multicenter observational study is to identify and characterize host and tumor-related biomarkers that can be used to distinguish expected responders from nonresponders for targeted therapy in mRCC. Here, we describe the cohort of recruited patients, their characteristics, and the collected data.

2. Material and methods

2.1. Study design and patient recruitment

EuroTARGET is an international, multicenter observational study that started in March 2011. The study was approved by the ethics committee at each participating center. A total of 748 patients were recruited prospectively in 62 centers; 36 in The Netherlands, 16 in Spain, 8 in Germany, 1 in Romania, and 1 in the United Kingdom. Patient identification and invitation procedures differed per country (Supplementary material), but at all locations, the following were the inclusion criteria: patients gave written informed consent, were at least 18 years of age, and had newly diagnosed metastatic renal-cell carcinoma. Patients were enrolled in the study regardless of RCC subtype and (pre)treatment.

In addition to the prospectively recruited patients, 462 patients with mRCC from previous studies ("historical patients") were included in EuroTARGET. Of these, 56 patients were retrospectively included in PERCEPTION, a Dutch population-based study with patients diagnosed between January 2008 and December 2010 [20]; 89 patients were enrolled between October 2007 and December 2010 by the Spanish Oncology Genitourinary Group [21]; 153 patients were included between June 2004 and October 2010 by a Dutch working group of 6 university hospitals focussing on sunitinib-induced toxicity [22]; and 35 patients were recruited to a study on the epidemiology and inheritance of renal cancer conducted by deCODE genetics in Iceland since 2001 [23]. The Radboud university medical center (Radboudumc) included another 66 patients, collected in a prospective biobank of patients diagnosed with cancer. The Central European Society for Anticancer Drug Research included an extra 63 patients, collected in prospective biobanks from Saarland University Medical Center in Homburg, Germany, Jena University Hospital in Germany, and University Hospital Graz in Austria. All biobanks were approved by the local ethics committees (Supplementary material).

2.2. Collection of clinical data

Clinical information from all patients was collected by medical file review and entered in web-based case record forms (CRFs) (Supplementary material and Table 1). All data were managed, exchanged cross-border, and used according to the data protection laws in Europe. Data included demographic information, baseline clinical characteristics, treatment lines, drug toxicities (Common Terminology Criteria for Adverse Events [CTCAE version 4.0] $\geq$ 3), tumor response (i.e., Complete remission, partial remission, stable disease, or progressive disease), and death. Tumor response was defined according to RECIST version 1.1 and based on patient evaluation by local caregivers as given in the radiology report or medical record (no review of imaging).

Table 1
Clinical information available in EuroTARGET web-based case record forms

Patient characteristics
Date of birth
Sex
Eligibility criteria
Date of diagnosis metastasis
General information at diagnosis
Height
Bilateral renal cell carcinoma
Tumor assessment at start of treatment
cTNM classification
pTNM classification
Comorbidities at start of treatment of metastasis
Treatment line
Clinical data at start of treatment of metastasis
Number of locations and location of metastases
Lab values at start of treatment of metastasis
Drug treatment
Dosing schemes
(Concomitant) nondrug treatment—surgery/radiotherapy
Toxicities
Responses
New lesions
Final information on patient
Date of death
Last registration date

cTNM = clinical tumor-nodes-metasasis; pTNM = pathological tumor-nodes-metasasis.

Clinical data were extracted from the web-based CRFs and checked for missing and inconsistent data. Both were resolved where possible by looking deeper into the CRF information or by going back to the medical files (Supplementary material). End points for efficacy and toxicity analyses, including progression-free survival (PFS) time, overall survival (OS) time, dose reductions, dose interruptions, and grade 3 or higher toxicities, were calculated from the clinical data using algorithms (Supplementary material).

2.3. Collection of biomaterials

Blood samples were collected from almost all patients for germline DNA isolation. In 12 German and Dutch centers, up to 12 plasma samples were collected per patient before and during sunitinib or pazopanib treatment, for measurement of drug and metabolite concentrations. In addition, tumor material from the kidney and normal kidney tissue was collected during nephrectomy if possible, and freshly frozen. This fresh-frozen material was mainly

collected in the German and Dutch academic centers. Paraffin-embedded tumor material from the kidney was not collected specifically for EuroTARGET, but it was collected from patients with histologically confirmed RCC at the local pathology departments. Slides were collected for central pathology review of tumor subtype and histopathological features by one of the four expert uropathologists from Spain, The Netherlands, Germany, and the United Kingdom. In The Netherlands, paraffin-embedded material was also used to construct tissue microarrays (TMAs) containing three 3 mm cores from representative tumor areas per patient. Blood or DNA samples or both and freshly frozen samples were coded and stored at the central EuroTARGET biobank at the Radboudumc, The Netherlands.

2.4. Platform and pharmacokinetic/pharmacodynamic analyses

EuroTARGET encompasses multiplatform omics profiling and pharmacokinetic/pharmacodynamic (PK/PD) analyses. Genome-wide germline DNA variation data were measured using Illumina HumanOmniExpress BeadChips. Tumor material was profiled using next-generation whole-exome sequencing (Illumina; tumor DNA and matched germline DNA), RNA sequencing (Illumina), PamChip kinase assays, and Illumina Infinium HumanMethylation450 BeadChips. PK/PD models were developed using NONMEM 7.3 software.

2.5. Availability of data

All clinical and platform data generated in EuroTARGET will be made freely available in an anonymized way for the research community as of March 2018. The data can be accessed through the European Genome-phenome Archive (EGA) which is the controlled access repository under the European Bioinformatics Institute (EMBL-EBI). Interested parties will be able to find the EuroTARGET project under the Studies section.

3. Results

In total, 1,210 patients with mRCC were included in EuroTARGET, of which 748 were collected prospectively and 462 were available from historical (prospective) series at the start of EuroTARGET. Of the 1,210 patients, we selected the 979 patients (81%) who received sunitinib, sorafenib, or pazopanib as first TKI (remainder of patients did, for example, have no treatment or were treated with an mTOR inhibitor or other TKI). Prior cytokine therapy was allowed. To enable informative future analyses, we only focus on the subset of 920 patients for whom outcome could be assessed for at least 6 months (24 weeks) (Fig. 1).

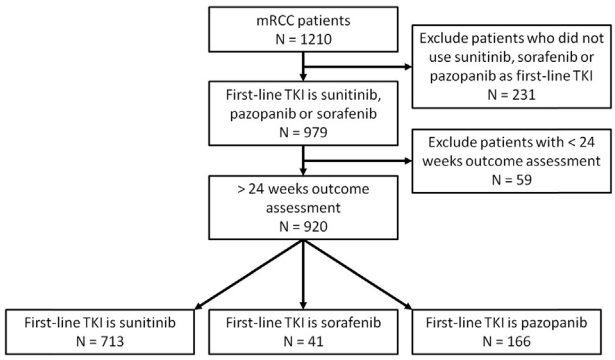

Fig. 1. Flowchart describing the selection of EuroTARGET patients for future analyses.

Table 2 displays the origin of the 1,210 patients collected for EuroTARGET and of the 920 patients who were selected as being relevant for future analyses.

3.1. Baseline characteristics

Baseline characteristics of the total patient population stratified by first-line TKI are shown in Table 3 (see Tables S1a and S1b in supplementary material for baseline characteristics

Table 2
Origin of the EuroTARGET patient population

	All collected patients $N = 1,210$		Patients to be included in analyses $N = 920$	
	Prospective recruitment	Patients from historical series	Prospective recruitment	Patients from historical series
The Netherlands				
EuroTARGET	264		150	
PERCEPTION	129	56	80	41
SUTOX		153		145
Radboudumc		66		46
Spain				
EuroTARGET	187		169	
SOGUG		89		87
United Kingdom				
EuroTARGET	52		45	
Romania				
EuroTARGET	33		19	
Germany				
EuroTARGET	83		60	
CESAR		55		37
Austria				
CESAR		8		6
Iceland				
DeCODE		35		35
Total	748	462	523	397

CESAR = Central European Society for Anticancer Drug Research; PERCEPTION = Dutch population-based registry of mRCC patients; SOGUG = Spanish Oncology Genitourinary Group; SUTOX = Dutch working group of six university hospitals focussing on sunitinib-induced toxicity.

Table 3
Baseline characteristics of the EuroTARGET patient population

	All patients $N = 920$	Sunitinib $N = 713$	Sorafenib $N = 41$	Pazopanib $n = 166$
Median age at start TKI, y	64	63	61	66
Range	20–87	20–87	45–82	40–84
Sex				
Male	663 (72.1%)	525 (73.6%)	32 (78.0%)	106 (63.9%)
Female	257 (27.9%)	188 (26.4%)	9 (22.0%)	60 (36.1%)
No. of metastases at start TKI				
1	323 (35.1%)	268 (37.6%)	12 (29.3%)	43 (25.9%)
2	324 (35.2%)	248 (34.8%)	16 (39.0%)	60 (36.1%)
3 or more	270 (29.3%)	195 (27.3%)	12 (29.3%)	63 (38.0%)
Unknown	3 (0.3%)	2 (0.3%)	1 (2.4%)	
Sites of metastases, *n* (%)				
Lung	634 (68.9%)	487 (68.3%)	33 (80.5%)	114 (68.7%)
Lymph node	394 (42.8%)	289 (40.5%)	22 (53.7%)	81 (48.8%)
Bone	258 (28.0%)	203 (28.5%)	8 (19.5%)	47 (28.3%)
Liver	163 (17.7%)	123 (17.3%)	6 (14.6%)	34 (20.5%)
WHO performance status				
0	323 (35.1%)	259 (36.3%)	11 (26.8%)	53 (31.9%)
1	347 (37.7%)	277 (38.8%)	7 (17.1%)	63 (38.0%)
2	48 (5.2%)	28 (3.9%)	1 (2.4%)	19 (11.4%)
3	7 (0.8%)	4 (0.6%)		3 (1.8%)
4	1 (0.1%)	1 (0.1%)		
Unknown	194 (21.1%)	144 (20.2%)	22 (53.7%)	28 (16.9%)
IMDC risk stratification				
Favorable risk	74 (8.0%)	65 (9.1%)	3 (7.3%)	6 (3.6%)
Intermediate risk	232 (25.2%)	188 (26.4%)	3 (7.3%)	41 (24.7%)
Poor risk	169 (18.4%)	134 (18.8%)	6 (14.6%)	29 (17.5%)
Unknown[a]	445 (48.4%)	326 (45.7%)	29 (70.7%)	90 (54.2%)
Prior nephrectomy	690 (75.0%)	534 (74.9%)	28 (68.3%)	128 (77.1%)
Cytokine therapy before TKI	54 (5.9%)	39 (5.5%)	14 (34.1%)	1 (0.6%)
Histology subtype[b]				
Clear cell	802 (87.2%)	620 (87.0%)	34 (82.9%)	148 (89.2%)
Non-clear cell	78 (8.5%)	66 (9.3%)	2 (4.9%)	10 (6.0%)
Unknown	40 (4.3%)	27 (3.8%)	5 (12.2%)	8 (4.8%)
Material available				
DNA	824 (89.6%)	650 (91.2%)	28 (68.3%)	146 (88.0%)
RCC tissue	142 (15.4%)	113 (15.8%)	15 (36.6%)	14 (8.4%)
Normal kidney tissue	95 (10.3%)	75 (10.5%)	8 (19.5%)	12 (7.2%)
TMA	247 (26.8%)	193 (27.1%)	16 (39.0%)	38 (22.9%)

TMA = tissue microarray; IMDC = International Metastastic Renal Cell Carcinoma Database Consortium; WHO = World Health Organization.
[a]IMDC risk stratification was unknown if one of the criteria to determine the risk was missing
[b]For histology subtype, we used the subtype defined by a central pathologist. If subtype was not determined by a central pathologist, we used subtype defined by a local pathologist. See Table S2 in the supplementary material for more information.

separately for the prospectively recruited patients and for the patients from historical series).

3.2. Follow-up data

Patient follow-up was completed up to March 1, 2016, and censored at this date if patients were still alive, which was the case for 241 patients (26%). A total of 478 patients (52%) were followed until their death. For 39 patients (4%), date of death was known, but follow-up was not completed up to the date of death. For another 162 patients (18%), follow-up information was not complete up to March 1, 2016, nor was date of death known. These patients were censored at the last date registered in the CRF. Median follow-up time from start of TKI treatment until death or censoring for all patients was 15.3 months (interquartile range [IQR]: 8.5–30.2 months).

Progression is defined as relapse or progressive disease, development of a new lesion, or death. We calculated PFS time as the time between start of TKI treatment and date of progression, death, or censoring (whichever happened first), regardless of duration of TKI treatment. OS time was calculated as the time between start of TKI treatment and date of death or censoring. A total of 681 patients (74%) experienced a progression event and 517 (56%) died during follow-up. Median PFS time was 10.8 months (IQR: 4.4–24.6 months) and median OS was 26.1 months (IQR: 10.6–51.3 months) (Fig. 2).

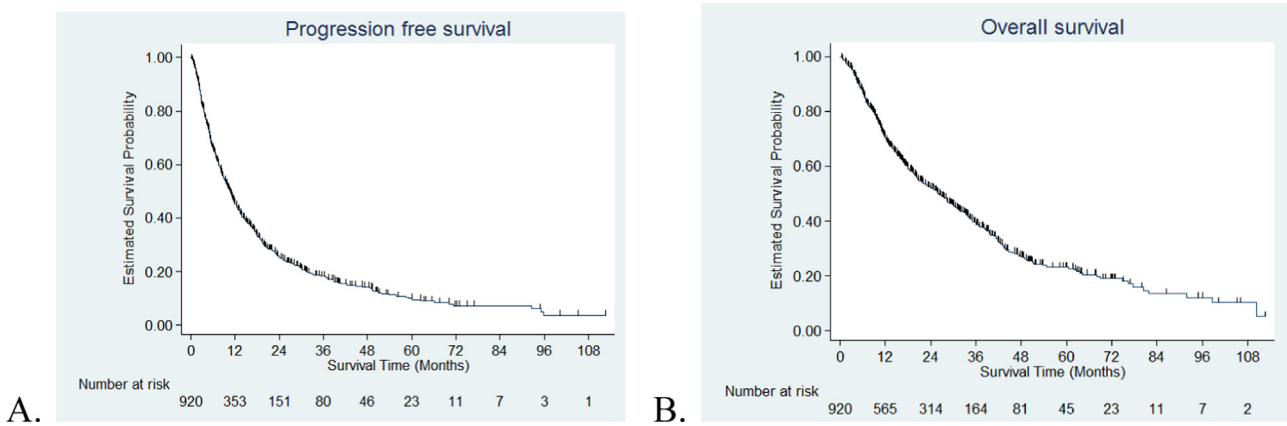

Fig. 2. Kaplan-Meier curves for progression-free survival (A) and overall survival (B). Vertical lines indicate censoring events.

We also evaluated PFS for the time period in which patients were actually using TKI treatment (sunitinib, sorafenib, or pazopanib). In this scenario, patients were censored when they stopped TKI treatment, and PFS time was calculated as the time between start of TKI treatment and progression, death, or censoring (whichever came first). With this definition, 546 patients (59%) experienced a progression event, and median PFS time was 11.7 months (IQR: 5.0–30.1 months).

Table 4 shows the follow-up characteristics of the total patient population and separately for the different first-line TKIs used (see Supplementary Tables S3a and S3b for follow-up characteristics for the prospectively recruited and historical patients).

3.3. Biomaterial and platform data

Germline DNA was available from 824 of 920 patients and fresh-frozen tissue from 145 patients (primary kidney tumor, $N = 142$; normal kidney, $N = 95$). TMAs were created from 247 patients. Platform data were successfully generated for the following number of patients (out of the 920): germline DNA variation chip data for 811, whole-exome sequencing data (tumor and germline DNA) for 80, RNAseq data (tumor mRNA and miRNA) for 124, processed kinase activity data for 121, and methylation data for 116 RCC tissues (and 23 normal kidney tissues). For 73 patients, all platform data are available. PK/PD data are available from 40 patients.

4. Discussion

EuroTARGET is a European collaborative project, which aims to discover and validate biomarkers to personalize treatment of patients with mRCC. Here, we describe the EuroTARGET patient population, a large, extensively phenotyped cohort of 920 patients with mRCC with most patients receiving sunitinib as first-line TKI. For most patients, genome-wide germline DNA variation data are available, making this the largest cohort for prognostic

Table 4
Follow-up characteristics of the EuroTARGET patient population

	All patients $N = 920$	Sunitinib $N = 713$	Sorafenib $N = 41$	Pazopanib $n = 166$
Follow-up until death				
Yes	517 (56%)	420 (59%)	24 (59%)	73 (44%)
No	403 (44%)	293 (41%)	17 (42%)	93 (56%)
Median follow-up time, mo	15.3	17.0	13.7	12.9
Follow-up until progression event				
Yes	681 (74%)	534 (75%)	32 (78%)	115 (69%)
No	239 (26%)	179 (25%)	9 (22%)	51 (31%)
Median PFS time, mo[a]	10.8	11.1	7.6	10.6
Follow-up until progression event while on TKI				
Yes	546 (59%)	441 (62%)	26 (63%)	79 (48%)
No	374 (41%)	272 (38%)	15 (37%)	87 (52%)
Median PFS time while being on TKI, months[a]	11.7	12.5	8.1	12.0

[a]Please note that in "Median PFS time," progression events after stopping TKI treatment are considered as events, even when time between stop of treatment and event was long. In "median PFS time while being on TKI," patients are censored at the moment they stop TKI treatment meaning that events that occur shortly after TKI treatment stop are not taken into account.

germline genetic biomarker studies in sunitinib-treated patients with mRCC. For 73 patients, germline genome, tumor genome, transcriptome, kinome activity, as well as methylome data are available, allowing for an integrated analysis of multiplatform data.

In recent years, a number of renal cancer biorepositories with extended platform data have been generated. For example, the International Cancer Genome Consortium (https://icgc.org/), including, among others, The Cancer Genome Atlas Project [24], has profiled more than 1,200 patients with renal cancer at the DNA, RNA, protein, and epigenetics level. These data have been very valuable for insight into the existence of molecular subtypes [25]. However, the number of patients with mRCC and clinical data is limited, restricting the value of these data for prognostic biomarker studies in mRCC. Indeed, potentially relevant prognostic biomarkers for mRCC have, to date, mainly been derived from (randomized) clinical trials [14,18].

In contrast to clinical trial biorepositories with strict inclusion and exclusion criteria, EuroTARGET is of an observational nature. Therefore, patients should be more reflective of the general TKI population and results better generalizable. However, it also has disadvantages such as the dependence on information that is registered in medical files for information retrieval. Also, more than 60 centers and 5 European countries were involved in patient recruitment, resulting in a number of challenges. For example, the start of patient recruitment was severely delayed because of difficulties in obtaining ethical approval for this observational study that was erroneously regarded as a clinical trial by many of the recruitment centers.

Inclusion of patients from historical series in EuroTARGET substantially increased the number of available patients. It also poses some concerns, as these patients were sampled for projects with different aims, in different time periods, and using different inclusion procedures. For instance, we observed a median PFS and OS time of 8.9 and 20.7 months in the historical patient series compared to 11.8 and 30.3 months in the prospectively recruited patients, possibly reflecting improvement in mRCC treatment over time. Although biomarkers can be identified regardless of this, the quantitative effect estimate of the biomarker may not be representative for all current patients with mRCC.

The importance of replication and validation of biomarker findings has been stressed in many publications [26]. The original objective of EuroTARGET was to perform a two-stage inclusion of patients and samples to allow for both a discovery and a replication cohort within the consortium. However, owing to recruitment difficulties, the distinction between discovery and replication cohort has been abandoned. Instead, we will use *external* mRCC patient cohorts for replication [27–29]. Also note that functional validation of biomarker findings using, for example, in vitro studies in established RCC cell lines are integral parts of EuroTARGET.

Currently, analyses of clinical and platform data (separately and integrated), PK/PD analyses, and functional studies are ongoing within the EuroTARGET consortium. We hereby hope to improve understanding of the critical molecular and resistance pathways involved in TKI therapy and to define new validated risk stratification criteria to be used in personalized mRCC patient management.

5. Conclusions

EuroTARGET is a European collaborative project including 920 patients with mRCC treated with sunitinib, sorafenib, or pazopanib. EuroTARGET data will be freely available from March 1, 2018. We hope that easy access will promote the uptake of EuroTARGET data by the research community, and thereby the progress in personalization of therapy for patients with mRCC.

Acknowledgments

We would like to thank all patients who participated and their treating physicians for inviting them.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.urolonc.2017.03.009.

References

[1] Ferlay JSI, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer, 2013;2013. Available at: http://globocan.iarc.fr. [accessed October 10, 2016].

[2] Aben KK, Luth TK, Janssen-Heijnen ML, Mulders PF, Kiemeney LA, van Spronsen DJ. No improvement in renal cell carcinoma survival: a population-based study in the Netherlands. Eur J Cancer 2008;44:1701–9.

[3] Gupta K, Miller JD, Li JZ, Russell MW, Charbonneau C. Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev 2008;34:193–205.

[4] Dabestani S, Thorstenson A, Lindblad P, Harmenberg U, Ljungberg B, Lundstam S. Renal cell carcinoma recurrences and metastases in primary non-metastatic patients: a population-based study. World J Urol 2016;34:1081–6.

[5] Yang OC, Maxwell PH, Pollard PJ. Renal cell carcinoma: translational aspects of metabolism and therapeutic consequences. Kidney Int. 2013;84:667–81.

[6] Choueiri TK, Motzer RJ. Systemic therapy for metastatic renal-cell carcinoma. N Engl J Med 2017;376:354–66.

[7] Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 2007;356:115–24.
[8] Thompson Coon J, Hoyle M, Green C, Liu Z, Welch K, Moxham T, et al. Bevacizumab, sorafenib tosylate, sunitinib and temsirolimus for renal cell carcinoma: a systematic review and economic evaluation. Health Technol Assess 2010;14:1–184. [iii-iv].
[9] Rini BI, McDermott DF, Hammers H, Bro W, Bukowski RM, Faba B, et al. Society for immunotherapy of cancer consensus statement on immunotherapy for the treatment of renal cell carcinoma. J Immunother Cancer 2016;4:81.
[10] Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M, et al. EAU guidelines on renal cell carcinoma: 2014 update. Eur Urol 2015;67:913–24.
[11] Heng DY, Xie W, Regan MM, Warren MA, Golshayan AR, Sahi C, et al. Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. J Clin Oncol 2009;27:5794–9.
[12] Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumdar M. Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol 2002;20:289.
[13] Heng DY, Xie W, Regan MM, Harshman LC, Bjarnason GA, Vaishampayan UN, et al. External validation and comparison with other models of the International Metastatic Renal-Cell Carcinoma Database Consortium prognostic model: a population-based study. Lancet Oncol 2013;14:141–8.
[14] Hsieh JJ, Chen D, Wang PI, Marker M, Redzematovic A, Chen YB, et al. Genomic biomarkers of a randomized trial comparing first-line everolimus and sunitinib in patients with metastatic renal cell carcinoma. Eur Urol 2017;71:405–14.
[15] Huang D, Ding Y, Zhou M, Rini BI, Petillo D, Qian CN, et al. Interleukin-8 mediates resistance to antiangiogenic agent sunitinib in renal cell carcinoma. Cancer Res 2010;70:1063–71.
[16] Deprimo SE, Bello CL, Smeraglia J, Baum CM, Spinella D, Rini BI, et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. J Transl Med 2007;5:32.
[17] Diekstra MH, Belaustegui A, Swen JJ, Boven E, Castellano D, Gelderblom H, et al. Sunitinib-induced hypertension in CYP3A4 rs4646437 A-allele carriers with metastatic renal cell carcinoma. Pharmacogenomics J 2017;17:42–6.
[18] Maroto P, Rini B. Molecular biomarkers in advanced renal cell carcinoma. Clin Cancer Res 2014;20:2060–71.
[19] Joosten SC, Hamming L, Soetekouw PM, Aarts MJ, Veeck J, van Engeland M, et al. Resistance to sunitinib in renal cell carcinoma: From molecular mechanisms to predictive markers and future perspectives. Biochim Biophys Acta 2015;1855:1–16.
[20] De Groot S, Sleijfer S, Redekop WK, Oosterwijk E, Haanen JB, Kiemeney LA, et al. Variation in use of targeted therapies for metastatic renal cell carcinoma: results from a Dutch population-based registry. BMC Cancer. 2016;16:364.
[21] Garcia-Donas J, Esteban E, Leandro-García LJ, Castellano DE, del Alba AG, Climent MA, et al. Single nucleotide polymorphism associations with response and toxic effects in patients with advanced renal-cell carcinoma treated with first-line sunitinib: a multicentre, observational, prospective study. Lancet Oncol 2011;12:1143–50.
[22] van Erp NP, Eechoute K, van der Veldt AA, Haanen JB, Reyners AK, Mathijssen RH, et al. Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity. J Clin Oncol 2009;27:4406–12.
[23] Gudmundsson J, Sulem P, Gudbjartsson DF, Masson G, Petursdottir V, Hardarson S, et al. A common variant at 8q24.21 is associated with renal cell cancer. Nat Commun 2013;4:2776.
[24] Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013;499:43-9.
[25] Chen F, Zhang Y, Şenbabaoğlu Y, Ciriello G, Yang L, Reznik E, et al. Multilevel genomics-based taxonomy of renal cell carcinoma. Cell Rep 2016;14:2476–89.
[26] Poste G. Bring on the biomarkers. Nature 2011;469:156–7.
[27] de Groot S, Redekop WK, Sleijfer S, Oosterwijk E, Bex A, Kiemeney LA, et al. Survival in patients with primary metastatic renal cell carcinoma treated with sunitinib with or without previous cytoreductive nephrectomy: results from a population-based registry. Urology 2016;95:121–7.
[28] Motzer RJ1, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J, et al. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med 2013;369:722–31.
[29] Low SK, Fukunaga K, Takahashi A, Matsuda K, Hongo F, Nakanishi H, et al. Association study of a functional variant on ABCG2 gene with sunitinib-induced severe adverse drug reaction. PLoS One. 2016;11:e0148177.