Final publishable summary report

Executive summary

Personalized medicine is envisioned as the next step forward in health care and potentially will change the standard of healthcare. To be able to truly personalize medicine, (tumour) profiles of patients must be defined that can guide treatment. The quest to find these so-called biomarkers has intensified because genetic information can now be analyzed at a reasonable cost. Because cancer is a genetic disease, this knowledge is pivotal for personalized medicine to guide treatment, to predict individual therapy response and resistance, ultimately leading to optimal treatment outcome while reducing unnecessary drug use and expense, the fundamental goal of personalized medicine.

Tyrosine kinase inhibitors (TKI), small molecules inhibitory against the tyrosine kinase portion of proteins, are now widely used in patients with metastatic renal cell cancer (mRCC). They show robust clinical effects and inhibit a broad spectrum of related receptor tyrosine kinases. Up to 75% of all patients experience some reduction in tumour burden and the median progression free survival (PFS) and overall survival has increased with about 6 months to approximately 1.5 to 2 years. The treatment is expensive with costs of about €4,000 per month per patient (life-long). Unfortunately, both response and toxicity are not predictable in the individual patient and drug choice, dose and sequence are highly empirical based on clinical parameters.

In EuroTARGET we aimed to identify and characterize host and tumour related biomarkers to predict responders and/or adverse responders from non-responders for targeted therapy in mRCC. The focus was at a number of high-throughput platforms, allowing a detailed view on the genetic make-up of the patients and their tumours and on enzyme activity levels.

To reach the EuroTARGET consortium's goal clinical and molecular data from a large homogeneous population of mRCC patients treated with TKI was collected. Genomic analysis showed that the response to the drug was associated with a certain genetic make-up of the patient. In addition, we showed that the risk to get RCC was also associated with a particular genetic make-up. Genetic analysis at different levels revealed that slight changes in proteins involved in cell growth might explain drug sensitivity and toxicity. Functional studies in model cells supported this observation. Enzyme activity demonstrated that different tumour enzyme levels may predict TKI efficacy. Moreover they may possibly be used to guide drug choice of the individual patient. Integrative analysis of these data is expected to reveal relevant (combinations of) biomarkers to define a final prediction rule that includes the clinical data and the identified markers. This needs to be transformed in an easy-to-use tool that can be used by treating physicians to predict the response and toxicity for mRCC patients.

Summary description of project content and main objectives

EuroTARGET is a European collaborative project on "TArgeted therapy in Renal cell cancer: GEnetic and Tumour related biomarkers for response and toxicity". It is funded by the European Commission under the Seventh Framework Programme (FP7). The project brings together 15 partner organisations (13 research institutes and 2 companies) from 8 European countries.

Each year more than 63,000 new cases of kidney cancer are diagnosed in the European Union. Surgery has been the hallmark of treatment and is effective for localized disease, but for the approximate 50% of all patients that have metastasized renal cell cancer (mRCC) at presentation or that develop metastases during follow-up survival has been extremely poor (5-10% 5-year survival). In the past few years, so-called targeted therapies that suppress the formation of tumour vessels (so-called anti-angiogenic drugs) have changed the clinical practice for patients with mRCC dramatically. These agents show remarkable effects and up to 80% of the patients have clinical benefit. Overall, progression-free survival (PFS) has increased by approximately 6 months. Nevertheless, responses are not durable and the use of sequential therapy is therefore becoming routine practice. Such sequential therapy provides clinical benefit by inducing tumour shrinkage and prolonged PFS survival in a large number of patients. With an increasing number of compounds becoming available, choice of compounds and sequence is becoming extraordinary challenging.

The response to the targeted treatments is highly variable ranging from (rare) complete remission to no response at all. Also, toxicity experienced by a substantial number of patients is highly variable. Unfortunately, both response and toxicity are not predictable in the individual patient. Drug choice, dose and sequence are highly empirical and thus far, the selection of targeted treatment for mRCC patients has been based on classical patient and tumour characteristics with relatively poor predictive ability. To improve therapeutic indices, avoid chronicity, prevent relapse, reduce adverse effects and permit greater cost effectiveness, advancements in the field of medicine are needed to offer solutions for diseases which are currently untreatable. To change to one-size-fits all model of the current standard of health care, personalized medicine is envisioned as the next step forward. Personalized medicine based on the (tumour) profile of patients could classify people into smaller subsets from one large disease group, in our case mRCC patients.

Advances in technology have accelerated such that genetic information from patients and tumours can be deciphered at a reasonable cost. This knowledge is pivotal for personalized medicine, particularly for cancer, which is a genetic disease. In this area of research we defined 3 essential components: clinical data and biospecimens collection, profiling of specimens, and data management, bioinformatics and prediction modelling to improve our understanding of the critical molecular and resistance and toxicity pathways involved and to define new risk stratification criteria to be used in personalized patient management.

The main objective of this project is to identify markers that predict response and toxicity of the currently used drugs by integration of data generated by multiple high-throughput platforms. To reach our objective clinical information of 1210 mRCC patients was registered and germline genetic profiling was completed on >800 blood samples to determine whether a particular genomic signature could predict clinical outcome. High-throughput next generation sequencing was performed to identify potential response markers based on heterogeneous RNA profiles. To study whether specific DNA-modifications, leading to up or down-regulation of specific genes occurred, the so-called methylome of mRCC was studied and correlated with clinical outcome. Finally, because the targets of the drugs are enzymes, we investigated whether the enzyme activity levels could predict drug response and/or toxicity. In functional studies we tested whether the former results could explain drug sensitivity at the molecular level.

Integration of all the data that have been generated on the level of DNA, RNA, and protein marker variation will allow the identification of combinations of DNA- RNA-protein markers that predict the response and toxicity to sunitinib more accurately. Moreover, separate analyses of these data may not reveal this information. These new validated risk stratification criteria to be used in personalized patient management should allow prediction of individual therapy response and resistance and will enable the monitoring of successful treatment outcome while reducing unnecessary drug use and expense.

The EuroTARGET project ran from March 1, 2011 until August 31, 2016.

Description of main S & T results/foregrounds

Patient recruitment, data collection

EuroTARGET is an observational study with the goal to discover and validate biomarkers for drugs that inhibit phosphorylation of specific proteins (tyrosine kinase inhibitor (TKI)) related to response of patients with metastatic Renal Cell Cancer (mRCC). Tyrosine kinases are enzymes that can transfer a phosphate group from ATP to a protein in a cell. It functions as an "on" or "off" switch in many cellular functions, e.g. in cell proliferation and response to external stimuli. In many cancer types this regulation is misbalanced, leading to unrestricted cell growth. Increased understanding of molecular mechanisms in Renal Cell Carcinoma (RCC) have led to the implementation of several TKI for the treatment of patients with metastasized RCC.

Sunitinib is currently the most prescribed TKI drug for mRCC patients. Other TKI that are used are pazopanib and sorafenib. After obtaining ethical approval for sample and data collection at each participating center a central electronic case report form (CRF) was developed for EuroTARGET in which extensive (clinical) data was registered including monitoring of TKI use, toxicity, progression free survival and overall survival. A user-friendly portal was established and data managers used this portal to access the CRF and register the patients on-line.

Patients with mRCC were recruited in The Netherlands, United Kingdom, Germany, Romania and Spain (748 patients in 62 centers). Additionally, historical series of mRCC patients from earlier studies were included: 462 patients recruited in The Netherlands, Austria, Spain, Germany, and Iceland. Thus in total 1210 mRCC patients were included (Figure 1). The vast majority of mRCC patients included (920) received a TKI as first treatment and had a follow-up of more than 24 weeks, a period deemed necessary to evaluate the effects of sunitinib, the most commonly prescribed TKI.

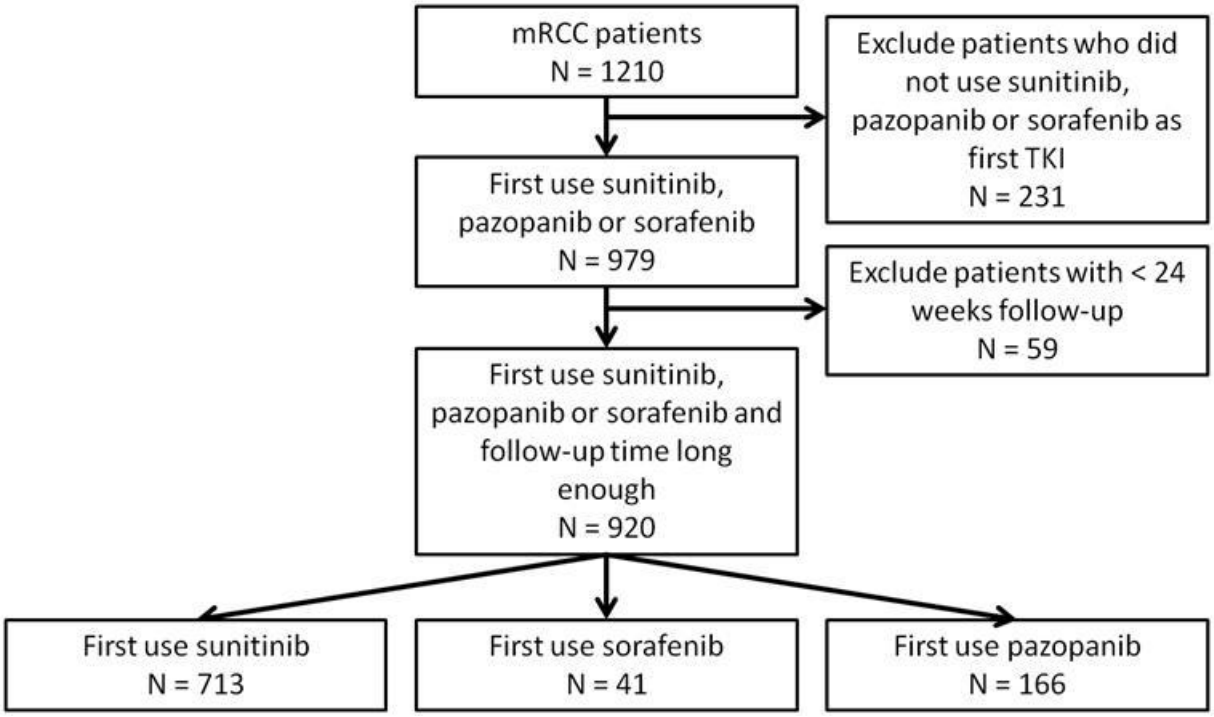

Figure 1: Flow diagram of EuroTARGET patients

Patient follow-up was censored at March 1st 2016. Median progression free survival (PFS), defined as the time between the start of TKI treatment and progression, death, or loss to follow up (whichever happened first), regardless of the duration of TKI treatment was 8.7 months.

Slides were collected for central pathology review by four expert pathologists from Spain, The Netherlands, Germany and the UK. For 683 of the 920 patients (74%), tumour subtype was reviewed by one of the four central pathologists. Reclassification to another RCC subtype was limited to 35 cases. Of the reviewed cases, 621 (91%) were defined as clear cell RCC (ccRCC), the most common RCC subtype, and 62 (9%) as non-ccRCC. This was as anticipated since sunitinib is first line treatment for ccRCC and in general patients with non-ccRCC receive another treatment. Of the 237 not reviewed by a central pathologist, 197 were seen by a local pathologist. For the remaining 40 patients, no information on subtype was available.

Material collection

Blood was collected and germline DNA samples are available from 824 patients. Fresh frozen RCC material was collected from 176 patients and fresh frozen normal kidney tissue was collected from 31 patients.

Additionally, RUMC received approval from the local ethics committee to collect paraffin embedded material from approximately 350 deceased mRCC patients of whom clinical data was already collected retrospectively. DNA was isolated from the paraffin embedded material, which will be used in the replication phase of the project to substantiate the validity of our findings. This paraffin embedded material was also used to construct tissue microarrays (TMAs) containing three 3 mm cores from representative tumour areas per patient. These will be used to validate potential sunitinib resistance/toxicity biomarkers at the protein level.

Data analysis

Clinical data were extracted from the EuroTARGET CRFs and checked for missings and inconsistencies. Cleaning steps were implemented and endpoints to be used in the 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. For all platforms identical clinical outcome parameters were used to be able to compare significant observations observed in different platforms and to be able to combine these. The clinical criteria to define good and poor outcome groups were based on response rate and overall survival. Follow-up characteristics are shown in Table 1.

Availability data

All anonymized clinical and platform data generated in EuroTARGET will be freely available for the research community as of March 2018, by downloading the datasets from an open repository. Blood samples and tumour material may be made available to other research groups, provided this does not conflict with informed consent, and after permission of all EuroTARGET partners involved in material collection.

Table 1. *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, months	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 regardless of TKI, months	8.7	8.8	6.5	8.7
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	7.4	7.6	4.3	5.9

PFS, progression free survival; TKI, tyrosine kinase inhibitor

Genetic profiling of mRCC patients

The genetic profiling of patients was performed to identify whether patients with a particular genetic make-up react differently to sunitinib treatment. In these analyses polymorphisms are tested at the single nucleotide level (so-called SNPs) with chips and such genome-wide association (GWAS) analysis have demonstrated associations between (a combination of) SNPs and disease outcome in other diseases. Here, we tested whether these germ-line genetic variants are associated with TKI treatment response and toxicity. We successfully genetically profiled 1,379 patients. After exclusion of patients for various reasons, for instance non-metastatic disease, data from a total of 572 patients were available. All 572 patients were included in toxicity analyses. For efficacy analyses, 65 patients with non-clear cell histology were excluded leaving 507 patients for efficacy analysis. Until now, the analysis was restricted to the association with progression free survival (PFS) and overall survival (OS). We identified 2 polymorphisms that were associated with PFS or OS. This means that we may be able to some extent to distinguish patients with a higher likelihood to respond longer and live longer with sunitinib treatment from other patients. The analysis to find associations between toxicity and genotype are currently being performed.

Due to the drastic reduction in the cost of chip genotyping that occurred from the time the application was written, it became clear that considerably more samples could be genotyped for the funds allocated to this part of the project. Therefore the genetic analysis was expanded to include a GWAS on the risk of RCC (both metastatic and localized disease). It is well established that risk of a particular disease can associate with a certain genetic make-up, but for RCC this has not been established. To this end, 282 non-metastatic RCC cases and 688 controls were genotyped. The results were combined with genotype data from a large number of RCC cases and controls collected by the consortium partners before the onset of EuroTARGET. We typed 1,501 RCC cases and 4,956 controls from The Netherlands; 91 RCC cases and 1,612 controls from Spain; 1,649 RCC cases, 287,706 controls from Iceland. Because different chips were used we used the homogeneous Icelandic population to filter out SNPs that showed different frequencies on different chips.
The meta-analysis of GWAS data from The Netherlands, Spain and Iceland yielded one novel locus that shows genome-wide significant association with risk of RCC. The strongest association is with a single nucleotide variant with frequency of 18% in European populations. Although our results suggest that this is a true association, we will seek to replicate variants in published RCC GWAS data.

For validation of our results, we will use GWAS data from other RCC series. The two main series are from Memorial Sloan-Kettering Cancer Center (MSKCC, New York, USA) and from the RIKEN Center for Integrative Medical Sciences (Japan). The MSKCC series has SNP genotyping available from 1099 RCC patients: 744 patients were treated with pazopanib, 355 patients received sunitinib. The studied patient population is similar to the EuroTARGET population. The RIKEN series has SNP genotyping available for 219 RCC patients. The study population is of different ethnicity, but otherwise comparable to our population. GWAS was performed to investigate possible association between germline variants and sunitinib toxicity. The coordinators of these studies have agreed to replicate our main findings in their sample sets. We will also be able to use the EuroTarget GWAS to validate their findings.

Transcription profiling of tumour material from mRCC patients.

DNA is translated to RNA which then serves as a template for a complex machinery, ultimately leading to the production of proteins. RNA levels are therefore important indicators of the cellular phenotype. In many cases different RNA levels have been used to stratify patients. To identify potential sunitinib-response markers on the RNA level, RNA sequencing (RNAseq) was performed on 177 samples. RNAseq uses advanced next-generation sequencing (NGS) methods to reveal the presence and quantity of RNA in a biological sample at a given moment in time. Initially we proposed to perform these studies with hybridization-based microarrays, even though we knew that there were technical drawbacks. However, the costs for RNAseq were at the time of writing the research proposal prohibitively high. Due to the substantial reduced costs of RNAseq we decided to perform gene expression analysis by RNAseq. In principle RNAseq accounts for all the transcripts in the cell. Differently expressed genes can be identified and these can be compared between samples, offering higher quality quantitative gene expression levels. Preliminary analysis of RNAseq data of a smaller group of patients suggested that sunitinib response of patients is correlated with specific mRNA and miRNA profiles. However, the group size was too small to reach a firm conclusion. To improve the statistical power of our analysis the results from all samples that have been analyzed were pooled. We aim to locate sets of genes that can be used as predictors for PFS and toxicity.

RNAseq data from 97 patients treated with sunitinib for whom all relevant clinical data were available were included. Based on the similarity of expression of 4500 genes patients could be divided in 2,3,4, and 5 clusters. The best division was achieved when patients were clustered in 3 or 4 clusters, similar to the gene signatures observed for clear cell RCC (ccRCC) by other investigators. Our results indicate that our population is similar to other cohorts with respect to molecular characteristics. Thus we are dealing with a typical population of patients with metastasized ccRCC. The results also show that we should be able to compare our results with publically available datasets.

Once the samples were grouped into clusters we related these clusters to the clinical end-points: PFS at 3, 6, or 9 months (Figure 1) and toxicity at 3,6,9 months of treatment. When patients were clustered in 3 groups, no correlation between PFS and gene signatures was observed (Fig. 1A). However, when patients were clustered in 4 groups a correlation between gene profile and PFS was observed (Figure 1B). There was no strong statistical correlation between the clusters and the endpoints but there was a trend. Interestingly, studies examining the metabolism of ccRCC has revealed the presence of four metabolic clusters with prognostic value. These four clusters were associated with the four reported gene expression clusters. Because we anticipate that (part of) the observed differences in efficacy and toxicity will be reflected by the metabolic profile it is reasonable to assume that for our analysis clustering in 4 groups is preferred.

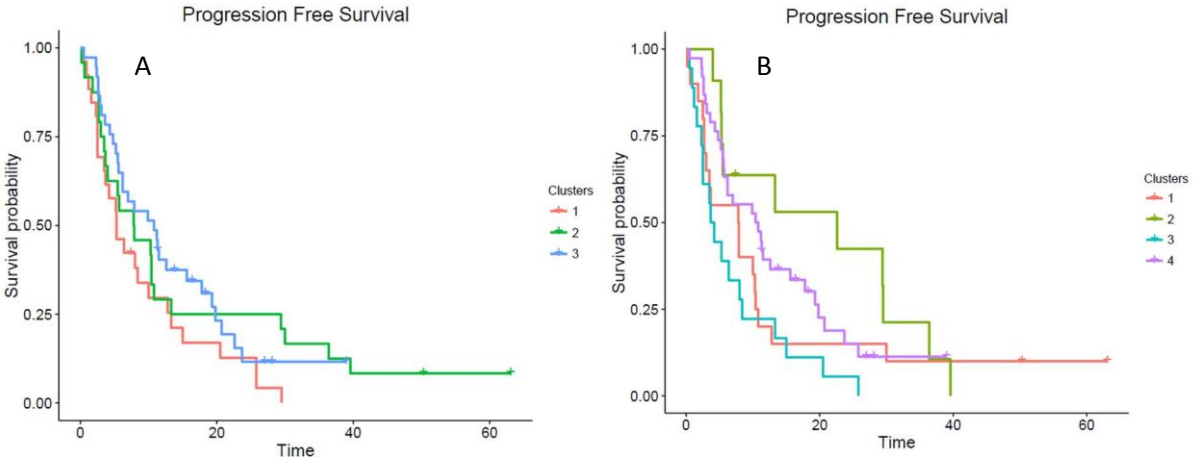

Figure 1: Progression free survival of EuroTARGET patient grouped in 3 or 4 gene signatures

When we examined whether a particular gene signature correlated with PFS at different time intervals sets of genes that correlated with progressive disease free intervals were identified. Four genes were identified that potentially associate with PFS. The proteins encoded by these genes are all involved in signalling events and pathways that are crucial for cell survival and cell proliferation. They act as activators or inhibitors of these processes. The results suggest that subtle changes in multiple genes involved in cell proliferation associate with sunitinib efficacy.

miRNA analysis

miRNAs are small RNAs of about 22 nucleotides in length that regulate gene expression. Our miRNA results also indicate that our population is similar to other cohorts with respect to miRNA molecular traits, again demonstrating that we should be able to compare our results with publically available datasets. Patients could be separated in 4 clusters based on their miRNA profile. There was no strong statistical correlation between the clusters and the predefined clinical endpoints but there was a trend.

In an alternative analysis we were able to show that PFS correlated with specific miRNAs. Several miRNAs that we identified have been implicated in cell proliferation. This fits well with the mRNA data that also show the importance of expression levels of proteins involved in cell proliferation. Interestingly, a relation with enzymes involved in drug metabolism was observed, suggesting that the sunitinib metabolism is related to fast or slower progressive disease of sunitinib-treated mRCC patients. Additionally, a relation with genes involved in cell migration was observed.

Further analysis. The identified sub-clusters are being compared to clusters defined by other investigators. Additionally, we are performing pathway analysis to pinpoint affected pathways driving sunitinib sensitivity, resistance and toxicity. Furthermore, common mutation profiles, and fusion gene profiles are being analyzed to study (recurrent) mutations and couple this information to PFS, OS and sunitinib response and toxicity. Similar to analysis of the subclusters we will use the databases mentioned before to validate our observations.

All cancers arise as a result of somatically acquired changes in the DNA of cancer cells. That does not mean, however, that all the somatic abnormalities present in a cancer genome have been involved in development of the cancer. Indeed, it is likely that some have made no contribution at all. To embody this concept, the terms 'driver' and 'passenger' mutation have been coined. 'Driver' mutations confer growth advantage on the cells carrying them. A number of so-called genes important for ccRCC have been identified. Interestingly, depending on which driver-gene is involved, patient outcome differs. Therefore it is important to know the driver-gene status of the RCC that we study. Although mutation analysis based on RNAseq results is possible, this is not trivial and it may not be safe to assume that a mutation occurred. Therefore whole-exome sequencing (WES) is being

performed on material from approximately 80 patients (normal and tumour DNA). In this method the genome of normal and tumour DNA is directly compared and this method is less vulnerable to false mutation calling. Once this analysis is complete patients will be divided according to the driver gene of the ccRCC and we will create survival curves to study their impact on survival in this homogeneous cohort of sunitinib treated patients. We should be able to determine whether a particular driver-gene signature correlates with PFS and/or OS. We will compare these cohorts with other established cohorts, albeit that those are contaminated with non-metastasized ccRCC patients.

In summary the gene expression data suggest that the sunitinib response of patients is dependent on subtle differences in expression of genes involved in cell proliferation and drug metabolism. This suggests that a specific gene signature may predict sunitinib response. With the addition of mutation data further stratification may be possible with the ultimate goal to personalize sunitinib use.

Kinase activity profiling of mRCC

In our joint effort of identifying biomarkers that can predict a patient's response to sunitinib, we used so-called 'peptide microarrays'. Using these miniaturized devices we were able to detect the kinases which are the targets of sunitinib. Tumour tissues of 183 patients with metastasized ccRCC were analyzed for enzyme (kinase) activities. The samples were profiled using 5µg of protein input (approx. 1/20 mm3 tissue) at 4 concentrations of sunitinib.
An interesting kinase activity profile weakly predicted sunitinib-response in a small set of samples. Unfortunately, this kinase activity profile was not predictive in the larger set. Identifying factors that may explain the differences between the training and validation cohorts is ongoing.
Response prediction after 3, 6 and 9 months of therapy was extended to analyzing the correlation between the kinase activity profiles with overall survival and finally by investigating the extreme survival groups (long versus short). The sunitinib response correlated (weakly) with OS and PFS in our analysis thus far.
The functional kinase activity readout was also used to assess the effects of other kinase inhibitors. We hypothesized that this might be predictive for therapy response. In a small sample set (4 versus 4), the sunitinib as well as axitinib inhibition correlated well with clinical response to sunitinib treatment. Other TKI (afatinib, cabozantinib, crenolanib and crizotinib), all with slightly different specificity, showed different responses for individual patients. This suggests that this assay might be used to individualize drug choice.

Significant higher kinase activities were detected in tumour tissues compared to normal tissues. Interestingly, differential kinase activities were observed between toxicity groups in tumour tissues and normal tissues. The NEK3 protein reporter site was more phosphorylated in the patient group experiencing higher toxicity levels. In tumour tissues higher kinase activities was observed in the patient group without toxicity. When normal kidney tissue was compared to tumour tissue higher kinase activity was observed in tumour tissues in patients without toxicity, while equal kinase activity was measured in the patient group showing toxicity.

A new assay was developed enabling phosphatase activity profiling. Phosphatases reverse the reaction of kinases and the balance between these two regulates important cellular processes such as cell growth and cell death. Thus, kinase and phosphatase assays may reveal subtle differences that explain TKI sensitivity. Proof of concept was shown in renal cancer samples, by detecting a correlation of high Protein Tyrosine Phosphatases (PTP) activity with high levels of tumour-infiltrated lymphocytes (TILs). The T-cell infiltration score itself is already regarded to be a candidate biomarker for Immuno-oncology therapy response. The observed correlation of phosphatase activity with lymphocyte infiltrates in renal cancer tissues is the basis for development of more mechanistic biomarkers predicting therapy response. This method may provide an alternative for the current semi-quantitative TILS quantification method.

DNA methylation biomarkers of mRCC

The main aim was to determine whether changes in DNA methylation patterns in genes and non-coding RNAs could be discovered for sunitinib response and toxicity of mRCC patients. Nucleic acid methylation modifications in an organism's genome or in a particular cell play an important role in gene expression. DNA methylation helps cells with gene regulation and methylome studies confirm a highly dynamic, yet tightly controlled, landscape essential to proper regulation of certain cell processes. It is therefore reasonable to assume that methylation patterns can (partly) explain sunitinib sensitivity. Furthermore, it is possible that methylation-regulated driver genes are associated with carcinogenesis in RCC. For this analysis more than 450,000 CpG methylation sites per sample were tested at single-nucleotide resolution, covering all genes. In total 96 mRCC and 31 normal kidney (NK) were used to perform methylome data analysis.
Methylome data analysis was performed to identify genes differentially methylated in association with sunitinib treatment outcome. The clinical criteria to define good and poor outcome groups were based on response rate and overall survival. We also studied whether methylation changes were associated with sunitinib-derived toxicity, but this did not yield any relevant result.
When we used very strict criteria, methylation changes were not associated with treatment. However, if we considered less restrictive criteria, we identified a set of genes related to sunitinib efficacy. A higher number of methylation changes (in 256 CpG sites, corresponding with 249 genes) was observed in patients who progressed versus non-progressive cases. For non-coding RNAs (ncRNAs) we identified 7 CpG positions from 2200 ncRNA transcription regions represented in the 450K array. Importantly, we also identified groups of CpGs that tended to show the same behaviour regarding methylation status.

Additionally, we aimed to identify driver genes regulated by methylation associated with carcinogenesis in RCC. We therefore compared a set of tumour samples with normal kidney (NK), and determining their role in RCC prognosis. We identified a large list of potential candidates (more than 200), where methylation changes were significantly associated with RCC carcinogenesis. We had previously observed a general hypomethylation in normal tissue compared with tumour, with a higher level of hypermethylation in promoter zones of the genome. We selected a list of the most relevant genes that showed remarkable methylation differences associated with RCC carcinogenesis. These genes were validated in an independent cohort. The results obtained in our cohort overlapped with the external cohort, indicating again their potential role in RCC carcinogenesis. However, none of them was associated with RCC progression-free survival. With respect to ncRNAs, we found remarkable differences in the methylation level of miR124.2 and mir124.3 between mRCC and NK, showing a gain of methylation in tumours. Again, however, we did not observe any difference between good and poor prognosis groups.

Summarizing, we identified a set of epigenetic biomarkers associated with carcinogenesis and response to sunitinib efficacy in a cohort of mRCC patients. Although these interesting candidates appear to be altered and may correlate with sunitinib efficacy, further studies in independent cohorts are warranted to validate their potential role as biomarkers in RCC management.

Functional studies

To verify whether gene expression signatures, methylation status and kinase status could explain sunitinib sensitivity functional studies were designed. Obviously, the specifics of the functional studies were highly dependent on the results of the platform studies. Unfortunately progress was slower than anticipated, mainly due to delay in patient recruitment, and therefore functional studies based on gene profiles identifying association with PFS or toxicity were also delayed.

Because results of functional assays should be immediately translatable to RCC it was important to define the most relevant cellular context to perform functional assays. It has recently been shown that the metabolic profile, drug response and transcriptome of many RCC cell lines is dissimilar to RCC. This underlines the importance of solid cell line studies to reach conclusions that are valid. For instance, gene silencing in a cell line that is not representative of mRCC may not provide valid results In preparation for the functional studies the intrinsic drug sensitivity (IC50) of various RCC cell lines as well as of primary RCC cultures was determined. We showed that RCC cell lines varied substantially in the sensitivity for sunitinib, N-desethyl-sunitinib (the active metabolite), axitinib, pazopanib, sorafenib and evirolimus.

To obtain cell lines with different sunitinib sensitivity multiple cell lines were cultured in the presence of sunitinib or N-desethyl-sunitinib. Sunitinib sensitivity altered significantly with the adapted cell lines proliferating at dose levels up to 20 µM sunitinib. For some cell lines the proliferation rate decreased significantly. These cell lines are ideal models for functional studies to understand the role of certain genes and gene expression levels in sunitinib resistance.

Because it became apparent that potential candidate genes that could be interrogated in functional assays would most likely need to be tested in pathway analysis, we performed RNAseq of the cell lines revealing their complete mRNA and miRNA expression level spectrum. This analysis showed that the cell lines differ in their relative expression profiles suggesting that they cover the complete expression spectrum of ccRCC. More importantly expression levels of genes implicated in sunitinib sensitivity differed, and therefore this spectrum of cell lines is sufficient for the foreseen studies.
Gene expression analysis revealed that DUSP1 (dual specificity phosphatase 1) expression levels correlated with progressive disease: patients with high DUSP1 expression showed slower progression. Interestingly, DUSP1 dictates the kinetics of activation of proteins directly involved in cell proliferation. It acts as a direct inhibitor (see figure 2). This correlation suggests that high DUSP1 levels, leading to lower activation and lower proliferation rates, may lead to tumours that are more susceptible to sunitinib treatment. Additionally, slower disease progression correlated with expression levels of other proteins involved in the pathway. Alternatively, high DUSP1 levels may be associated with slower proliferative tumours, i.e., higher levels are related to intrinsic slower tumour growth and not to sunitinib response.

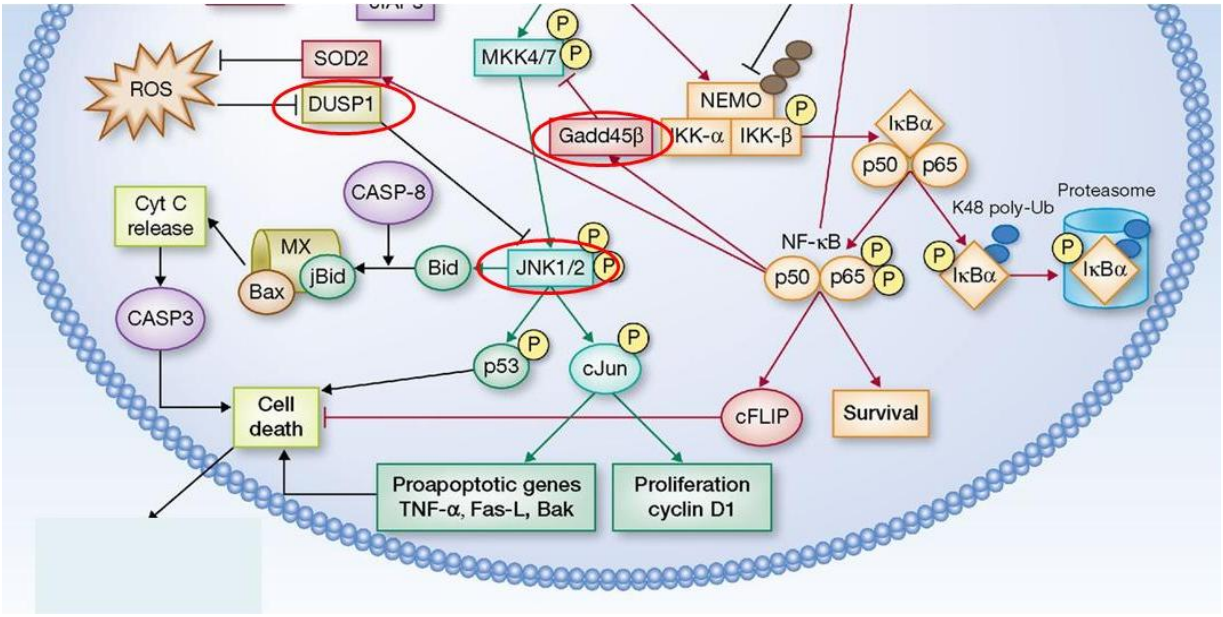

Figure 2: overview of pathways involved in cell death and proliferation. Differentially expressed genes related to sunitinib response/PFS are shown in red circles.

In view of the pathway convergence of the genes involved in sunitinib response, RCC cell lines (sunitinib resistant and parental) were treated with sunitinib or N-desethyl-sunitinib and tested for downstream target proteins. We observed that sunitinib treatment directly interfered with signalling. The cell line results demonstrated that not all cell lines will reflect RCC and therefore cell lines will be selected carefully to test the influence of e.g. DUSP1 interference on sunitinib sensitivity. We are currently introducing genetic modifications in the cell lines to test the relevance of target gene expression levels and their association with sunitinib sensitivity.
To identify kinase activity levels in cell lines with acquired resistance to sunitinib versus the sensitive parental cell lines were investigated. Remarkably, in general kinase levels were not altered in the sunitinib-resistant lines.

Because various proteins involved in cell growth signaling are regulated by serine-threonine phosphorylation, we investigated the level of serine-threonine kinase levels using a newly developed assay. Elevated serine-threonine kinase activities were observed in cell lines that were made sunitinib resistant. These findings need further study. They suggest that part of the sunitinib-resistance is related to altered serine-threonine kinase levels (non-sunitinib targets). Indeed, RNAseq studies (see earlier) showed the importance of expression levels of genes that are regulated by serine/threonine phosphorylation and a disbalance could explain sunitinib sensitivity.

Analysis of the effects of knock-down or up-regulation of candidate genes is still ongoing. In order to investigate the function and role in signalling pathways of identified mRNAs and miRNAs we are using sophisticated protein determination techniques. We analysed serum samples from patients treated with sunitinib or pazopanib. For patients progressing at 3 months (essentially sunitinib-resistant RCC) compared to the clinically responding patients (SD and PR), this analysis revealed 4 differentially expressed proteins between patients with progressive disease and patients with response. Similarly, for patients progressing at 6 month this analysis showed different serum levels of proteins. The results suggest that non-responder patients can be identified based on a serum protein profile. Thus, alternative treatment strategies might be considered. Because the results were obtained in a limited number of patients validation of these findings need to be confirmed in a larger patient cohort.

We performed microarray analysis on parenteral and Sunitinib resistant cells from 2 cell lines in order to identify micro-RNAs associated with Sunitinib resistance in vitro. We defined a miRNA signature associated with sunitinib-resistance. We are using these wild type and resistant cells to define resistance associated miRNAs in comparison with tumour tissue investigations.

In order to elucidate the autonomous response of cancer cells to sunitinib treatment in vitro, we analysed cancer cell motility upon treatment with Sunitinib. Sunitinib-resistant cells migrated significantly slower than the parental counterparts. The expression of proteins involved in cell movement and adherence was increased in the drug-resistant cells under the untreated conditions and in both cell subpopulations upon treatment with Sunitinib. This protein expression analysis supports our data on the migration-inhibiting impact of Sunitinib since it is well established that the increased expression of the identified proteins are intimately connected and vital for cell adhesion and migration.

Pharmacokinetic / pharmacodynamic modelling

The probability that a patient responds to a targeted drug therapy or develops toxicity is considerably influenced by the individual exposure to the drug and possibly active metabolites. Therefore, a pharmacokinetic /pharmacodynamic (PK/PD) model was further developed. In this technique the two classical pharmacologic disciplines of pharmacokinetics and pharmacodynamics

are combined. Pharmacokinetics (PK) determines the fate of substances (such as drugs) administered to a living organism and it studies how an organism affects a drug, whereas pharmacodynamics is the study of how the drug affects the organism. Both together influence dosing, benefit, and adverse effects, as seen in PK/PD models. For instance, patients with slow drug metabolism will reach higher drug levels then patients with faster drug metabolism. For the PK/PD study, 44 patients (27 receiving sunitinib, 17 receiving pazopanib) were recruited.

Sunitinib

For PK/PD analysis the newly acquired data for mRCC patients was pooled with data from a previous study in which metastasized colorectal cancer (mCRC) were treated with sunitinib. This allowed us to obtain a more precise model and improved the reliability of the analysis. A published PK base model was successfully adapted to the EuroTARGET study population. Pharmacokinetic differences between mRCC and mCRC patients were not identified. Furthermore, none of the tested variables that could influence the drug kinetics showed a significant impact on the model parameters. However, higher baseline values of sVEGFR-3 were observed in mRCC patients compared to mCRC patients

Based on serum levels of VEGFR-2 and VEGFR-3, both targets of sunitinib, the model was able to describe the sunitinib concentration in time in mRCC and mCRC patients. Two SNPs correlated with decreased intrinsic activity of sunitinib. Thus, genotyping of patients for these 2 SNPs may lead to patient stratification and in higher drug dose administration to reach higher serum drug levels for patients with decreased intrinsic sunitinib activity. In the small population, genotypes, sunitinib pharmacokinetics, sVEGFR-3 and blood pressure did not show a statistically significant effect on time-to-progression.

We modelled the two most common toxicities fatigue and myelosuppression (including anemia, thrombocytopenia, panzytopenia and neutropenia). For myelosuppression the inclusion of the active sunitinib/SU12662 concentration and the presence of a more active drug metabolism correlated with a higher risk of adverse events. However, no such correlations were found for fatigue. Thus, myelotoxicity is more likely to occur in patients that can be defined based on active drug concentrations in the blood and a defined genotype. The use of such a combination might allow fine tuning of drug dosis to prevent myelotoxicity.

Pazopanib

A structural model for pazopanib was built based on a recently published population pharmacokinetic model. The model was applied to the EuroTARGET data. In a final step biomarker response will be analyzed as predictor for clinical outcome based on a time-to-event model.

Data management

The massive data becoming available and the need to provide multiple partners access to these data justified the development of a separate ICT-based solution. The DiseaseMiner (DM) system was developed so that it could be used by all the partners in the project. Importantly, DM was accessible to partners over the internet, providing access to large datasets at the patient level. Data from the various partners was adapted to use in the DM. The system was used by the partners to browse the database and join data from various sources using simple queries. Although it is possible in theory to use the system to create any extract from the data, it turned out to be impossible to use the system directly for filtering and transforming data into datasets that can be used in genetic analysis. This was partially due to the fact that the queries needed for this were prohibitively complicated. Therefore, the system does not outperform traditional methods in making such datasets. Despite this, after generating such sets outside the system using traditional methods, the sets were also imported into the system for browsing and joining with other data.

Integrated data analysis

Currently, treatment guidelines for mRCC prescribe the use of two tools to predict prognosis in mRCC: the Memorial Sloan Kettering Cancer Center (MSKCC) and the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) models. These models are based on combining values for a number of clinical parameters (performance status of the patient ('general well-being'), delay between diagnosis of RCC and start of treatment, LDH value, corrected calcium value, haemoglobin value, neutrophil count, and platelet count). Verification of the accuracy and usefulness of these models for predicting prognosis in large populations of patients that are treated with sunitinib is important; these tools are namely used by oncologists to select a treatment strategy. Analysis of the two models in clinical data of 563 EuroTARGET patients showed that indeed both models can be used to distinguish patients with a good, intermediate and poor prognosis in terms of overall survival and time to progression of the disease. The IMDC slightly outperforms that of the MSKCC model. Our study also showed that for many patients the relevant clinical information for the MSKCC and IMDC models are not written down in the medical files by the treating physicians.

A simultaneous analysis of all the data that have been generated on the level of DNA, RNA, and protein marker variation will allow the identification of *combinations* of DNA- RNA-protein markers that predict the response and toxicity to sunitinib more accurately. Moreover, separate analyses of these data will not reveal this information. These integrated analyses are currently in progress.

As soon as the relevant (combinations of) markers have been identified by these so-called integrative analyses, a final prediction rule that includes the clinical IMDC model and the identified markers can be evaluated. It is important to note that the current analyses have shown relatively weak signals and it is unclear whether incorporation of our findings in the current models will improve the predictive value. Depending on univariate and multivariate analysis we aim for the transformation of this rule into an online, easy-to-use tool that can be used by treating physicians to predict the response and toxicity for sunitinib-treated patients.

Training and dissemination

A project homepage was developed (www.eurotargetproject.eu) to inform the public and to foster communication of consortium members within a restricted area. Open workshops were dedicated to provide training to senior and junior team members in the participant organizations and included technique-specific, scientific and ethical topics. Workshops were organized for clinical investigators involved in the collection of clinical data to address operational and logistical questions and to provide clinicians involved first-hand information on the results of the project.
The consortium disseminates scientific findings to the scientific and medical community in scientific meetings and congresses with a specific focus to oncologists and urologists

A Public-domain web-based Predicted Response and Toxicity Calculator (PRTC) will be developed that can be used by physicians to apply a personalized medicine approach to their patients. The final algorithm is not yet available for implementation. Nonetheless, all necessary preparations have been met in cooperation with the third-party provider to prepare the implementation once the final algorithm becomes available. Obviously, we can only strive for implementation if the PRTC is superior to the currently available algorithms that are solely based on clinical data. Currently, analysis of the individual high-throughput platform has not (yet) revealed very strong biomarkers associated with sunitinib response. Provided the PRTC becomes available, we will involve the European Association of Urology (EAU) and the European Society for Medical Oncology (ESMO) to advance the use of this Calculator in the medical profession. In addition, we will use measures to promote the PRTC via a workshop with European opinion leaders in the field of medical oncology.

Ethics

The ethical aspects were carefully managed through an ethics policy that took into account the specificities of the project. In addition the EuroTARGET consortium was active in several public consultations regarding regulatory and ethical dimensions (e.g. EMA , World medical association), participated in several actions regarding the General Data Protection Regulation and organized debates and presentations regarding ethical dimensions during the annual EuroTARGET consortium meetings (on sequencing in medical practice, on biomarkers, on data protection evolving regulation and on data sharing).

Potential impact and main dissemination activities and exploitation results

Potential impact

Cancer remains an important public health problem in Europe for patients themselves, their family as well as health care systems across Europe. Improvement of human health and quality of life and advancements in the field of medicine are needed to offer solutions for diseases which are currently untreatable. Personalized medicine is envisioned as the next step forward in health care and potentially will change the standard of healthcare. It will eliminate the "one-size fits all" methods widely accepted today. Advancements in personalised medicine are needed to improve therapeutic indices, avoid chronicity, prevent relapse, reduce adverse effects and permit greater cost effectiveness. Figure 3 shows how personalized medicine could classify people into smaller subsets from one large disease group. Advances in technology have accelerated such that genetic information from patients and tumours can be deciphered at a reasonable cost. This knowledge is pivotal for personalized medicine, particularly for cancer, which is a genetic disease.

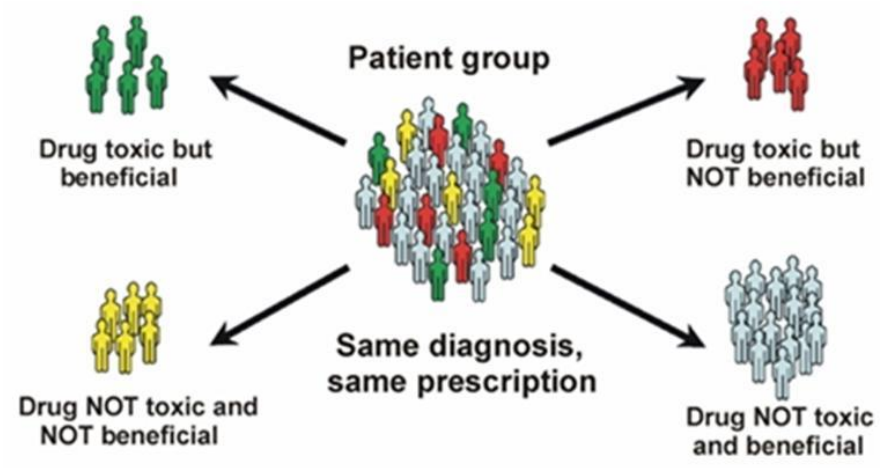

Figure 3: Patient stratification of patient with the same diagnosis can lead to better therapy outcome with less toxicity.

To reach the goal of personalized medicine, new validated risk stratification criteria to predict individual therapy response and resistance leading to optimal treatment outcome while reducing unnecessary drug use and expense are therefore urgently needed. In some cases therapies are already selected based on genetics and the biology of the tumour. For instance, melanoma can be BRAF positive, meaning the tumour has a specific gene mutation that sets it apart from other melanomas. For lung cancer EGFR and ALK alterations are important. Based on this information therapy is already being tailored along the lines of the genetic make-up of these tumour.

Kidney cancer is the 10th most common cancer in the European Union with more than 64,000 newly diagnosed patients yearly and more than 27,000 deaths. Surgery is quite effective for localized disease. However, for the approximately 50% of patients with metastasized RCC, 5-year relative survival was only 5-10%.

Knowledge of underlying molecular characteristics provided a rationale for targeting growth factor signaling pathways in RCC. Multiple tyrosine kinase inhibitors (TKI), small molecules that act against the tyrosine kinase portion of proteins are now widely used in clinical practice. Because these agents inhibit a broad spectrum of related receptor tyrosine kinases (i.e. are more general tyrosine kinase inhibitors or TKIs), they also differ in the spectrum of inhibitory effects and potency against any single receptor. These and other targeted agents have shown objective response rates up to 45%, twice as high as previous standard treatment. 70 to 75% of all patients experience some reduction in tumour

burden and the median progression free survival and overall survival has increased with about 6 months to approximately 1.5 to 2 years. Thus, targeted therapy in mRCC can be considered a revolution after decades without any progress. Unfortunately, the treatment is expensive with costs of about €4,000 per month per patient (life-long) which means that the drugs are not available in a large part of the EU, particularly in the former Eastern-European countries.

Even though the new drugs are 'targeted therapy', aiming at specific pathways, not all patients show clinical benefit from therapy, and inherent or acquired resistance to the drugs poses a problem. Sequential therapy is therefore becoming routine practice. With an increasing number of compounds becoming available, however, choice of compounds and sequence is becoming extraordinary challenging. In addition to the highly variable clinical response to the targeted treatments, toxicity, experienced by a substantial number of patients, is highly variable and frequently necessitates dose reduction or even cessation of therapy. Unfortunately, both response and toxicity are not predictable in the individual patient. Therefore drug choice, dose and sequence are highly empirical. The fundamental question facing the medical oncologist caring for a mRCC patient is how to consider the available agents and data to formulate an evidence-based individualized treatment approach. Until now, treatment choice in mRCC is determined by clinical parameters and based on these parameters, patients are stratified into a good, intermediate or poor risk group. Treatment choice is partly based on this risk grouping. Although this risk grouping has clear prognostic value it is very clear that the grouping is far from optimal: the agents lead to different clinical effects in different patients.

EuroTARGET aimed to identify and characterize host and tumour related biomarkers and to predict responders and/or adverse responders from non-responders for targeted therapy in mRCC. The overall concept was to focus on germline genome and tumour transcriptome, methylome and kinome-related biomarkers using an hypothesis-free and integrative approach and to evaluate promising findings via replication as well as functional assays.

The EuroTARGET consortium has collected clinical and molecular data from a large homogeneous population of mRCC patients treated with TKI. Additionally, RCC material and normal kidney was collected from patients. Furthermore, tissue microarrays (TMAs) containing three 3 mm cores from representative tumour areas per patient were created from tissue blocks of non-EuroTARGET patients. This repository will be valuable for the research society interested in RCC. This collection will be valuable for the research community at large and therefore the information will be shared after anonymizing patient data.

During implementation of EuroTARGET we learned that, depending on the country where data and/or materials were collected, policies varied substantially. Although patients were observed only, and clinical management of a patient was not influenced by inclusion in EuroTARGET, in many cases local ethical committees still wanted to individually change and approve the study. Apparently these committees viewed this study as an interventional clinical study. This shows that harmonization (also within EU countries) is poor and needs to be improved.

Protecting patient privacy and secure handling of data were regarded as important aspects that should not be underestimated. Therefore secure ICT measures were developed to arrange central registration and monitoring. Moreover, a central browseable database was developed that contained data from multiple platforms that was only accessible to predefined individuals. This interface can serve as an example for similar projects.

The large EuroTARGET population allowed us to study the value of currently used models for ccRCC patient stratification based on clinical information. The analysis of the clinical data in EuroTARGET confirmed the usefulness of the currently used MSKCC and IMDC models but also highlighted the lack of reporting of these variables in medical files.

Genomic analysis of the EuroTARGET patients showed the possible association between 2 polymorphisms that were associated with PFS or OS. These polymorphisms are dissimilar to polymorphisms in VEGFR3 and IL8 identified in earlier studies to be predictive for TKI response. The analysis to find associations between toxicity and genotype in EuroTARGET patients are currently being performed. Polymorphisms in the germline DNA have already suggested a significant role for allelic genotypic differences that are correlated with toxicity and our study should be able to validate their findings. Through validation of all polymorphisms, we will be able to determine the value of these polymorphisms in predicting response and we may be able to distinguish patients with a higher likelihood to respond longer and live longer with sunitinib treatment from other patients by a simple genomic analysis.

The availability of high quality primary tumour material from a large group of patients allowed gene expression, gene methylation and kinase studies. In general, such studies are restricted to cell lines and xenografts models, which may differ significantly from ccRCC from patients. Gene expression analysis showed that our patient population correlates to gene expression profiles derived by others. More importantly, we were able to associate a number of differentially expressed genes with PFS. Some of the identified genes are part of a common cell signalling pathway, leading to enhanced cell proliferation. From a biological point of view this means that highly proliferative cells are more likely to respond to sunitinib therapy. This results could partly be substantiated in cell line studies which showed remarkable differences in protein expression studies of sunitinib-resistant and sunitinib-sensitive cell lines.

Gene expression studies of miRNA suggest strongly that differences in expression of genes involved in drug metabolism and cell migration are associated with sunitinib sensitivity. The observation that regulation of expression of one of the drug metabolism gene products is associated with sunitinib sensitivity shows that most likely levels of this miRNA directly influence sunitinib metabolism. This is then directly reflected by the fast or slower progressive disease of sunitinib-treated mRCC patients.

The methylation studies also revealed methylation patterns associated with PFS. Interestingly, one interesting candidate (not identified in the gene expression analysis) has been reported to be related with cancer progression. In our study, methylation changes in this gene are associated with PFS.

The collective evidence of the gene expression and methylome studies show that sunitinib-response is connected to subtle changes The results suggest that subtle changes in multiple genes involved in cell proliferation, cell migration, and drug metabolism. In view of the sunitinib targets, this may not come as a surprise as most signalling pathways are governed by phosphorylation events. However, real time data from a large cohort of mRCC patients has not been presented thus far, and our increased understanding may enable patient stratification based on a combination of the identified biomarkers.

High-throughput screening of ccRCC has been very limited, although kinomic profiling of ccRCC may assist in devising a classification system associated with clinical outcomes, and help identify potential alternative therapies. The available RCC tumours were profiled for kinase activity but it was not possible to predict sunitinib response of patients. Thus patients could not be stratified. However, kinase activity inhibition assays did correlate with sunitinib response. Moreover, the inhibition assays also showed that sensitivity for different TKI differed between patients, suggesting that such assays can be used to guide drug choice. Furthermore, kinase activity could be correlated to toxicity. Thus, kinase assays could become a valuable tool to guide drug choice and prevent toxicity, leading to a more personalized approach for mRCC patients.

Although genomic profiling, gene expression studies, methylome studies and kinase studies can be informative and associations between sunitinib and PFS and OS can be determined, it is equally important to understand drug availability. Sunitinib needs to be converted to the active drug and

more than 99% of the drug is serum-bound and not active. Consequently, slight differences in drug metabolism can already have a dramatic effect on availability of the active drug. So-called fast metabolizing patients may need a different sunitinib dose compared to slow metabolizing patients for which lower dose may be as effective but prevent toxicity due to too high drug dosis. EuroTARGET developed models that can guide future studies which aim at identifying predictive biomarkers for efficacy and toxicity taking their time course in consideration.

Integrative analysis of biological data on DNA, RNA and proteins is currently ongoing. Obviously, this will contribute to increased insight into biological mechanisms that play a role in the prognosis of mRCC in patients that are treated using sunitinib. Ultimately, the incorporation of identified biological markers of response and toxicity into existing clinical prediction models, should improve prognostication and guide the making of the prediction tool. Clearly, the value and impact of this tool heavily depends on the added value over the current tools that exclusively use clinical parameters. This tool will be made freely available as an online webtool provided that it increases the possibility to predict sunitinib response and toxicity of mRCC patients beyond the current models to optimize exploitation and thereby maximize potential impact on clinical practice. Sharing and exploitation of the results will also take place via common scientific channels, including presentations at relevant conferences and meetings and publication in scientific journals.

The ethical aspects were discussed in several forums and debated in specific workshops (2012) and Conferences specialized in cancer (such as ICGC meetings in 2014 and 2016 Bejing, Boston) or oriented on values and societal aspects (such as the Conference “Sharing science, sharing values” in December 2015 in Toulouse). The survey conducted on views on large scale genomic exploration of professionals and patient associations is giving an impact on societal dimension and insights on the various actors views.

Main dissemination activities

Since the majority of the results arrived at the end of the project, the peak in dissemination activities will take place after the end of the project. Results will be disseminated via:

- The EuroTARGET website
- The websites of the partner institutes
- publications in medium/high impact scientific journals, specific for cancer and medical research
- oral presentations or posters at scientific events such as AACR 2017, ASCO June 2017, EAU Meeting March 2017 (late breaking abstract), the yearly meeting of the Dutch Society of Epidemiology (WEON) in June 2017, Meeting of the German Society of Urology (DGU) September 2017.
- presentations on contact days of patients’ organisations
- In 2017 we will organise a conference open to a broad public, to disseminate the results of EuroTARGET.
- We will propose a EuroTARGET session at the Euroscience Open Forum, held in Toulouse, France on 9-14 July 2018 (call for sessions will be in January 2017).

Exploitation results

The EuroTARGET project generated a large number of results that advance general knowledge in the field of oncological research:

- The clinical data will be anonymized and coupled with genetic data and will become available for the research community at large.
- Material collected during EuroTARGET (DNA, tissues, TMA, RNA) will be made available to the research community
- GWAS data will be used by other GWAS studies to validate potential associations
- Gene expression results and mutation analysis of the largest number of patients with metastatic RCC thus far worldwide.
- A new peptide microarray product was developed, now available for use in cancer research
- A new test and methodology was developed: On-Chip-Pharmacological profiling, permitting multiple drug testing.
- A new method to integrate multimodal (enzymatic and genomic) test results
- Supportive data for a patent in exploitation of predictive biomarkers in immunotherapies was generated
- A patent application will be filed of the first sets of candidate biomarkers that can be used for targeted therapy in urologic oncology
- A set of epigenetic biomarkers associated with carcinogenesis and response to sunitinib efficacy was discovered in a cohort of mRCC patients.
- Potential candidate genes were identified (more than 200), where methylation changes significantly associated with RCC carcinogenesis.
- Panel of cell lines with distinct sunitinib-response profiles and corresponding gene expression profile that can serve as models for functional studies.
- PK/PD models suitable to apply to data from other studies to extend the modelling framework even further (e.g. by testing other adverse events or adding tumour growth models).
- A database containing clinical and omics data for mRCC patients. An interface to browse the database. A web based system for querying and joining heterogeneous data on subjects.
- Generation of a novel prediction tool for mRCC patients.
- Precision medicine through integration of data obtained from different (high-throughput) platforms.

Contact information

For more information about the EuroTARGET project, please contact the coordinators:

Prof. dr. Lambertus A. Kiemeney
Radboud University Medical Center
Department for Health Evidence 133
PO Box 9101, NL-6500 HB Nijmegen, the Netherlands
Email: bart.kiemeney@radboudumc.nl
Phone: +31 24 3613745 / +31 24 8186805
Fax: +31 24 3613505

Dr. Egbert Oosterwijk
Radboud University Medical Center
Department of Urology 267
PO Box 9101, NL-6500 HB Nijmegen, the Netherlands
Email: egbert.oosterwijk@radboudumc.nl
Phone: +31 24 3614907
Fax: +31 24 3541222

or visit the EuroTARGET website:
www.eurotargetproject.eu