TRANSCAN's success stories from 3 projects funded under the Second Transcan Joint Transnational Call 2012: “Translational research on primary and secondary prevention of cancer”

SHORT INFO ON JTC 2012:

Participation of 17 ministries and agencies, supporting research and innovation activities in the field of translational cancer research from Europe and beyond. The Call aimed at developing transnational innovative projects in cancer prevention, focused on the research of the mechanisms responsible for maintaining a healthy status vs. those underlying cancer development, and clearly oriented towards a rapid translation of the existing and newly acquired knowledge into individual- or patient-tailored interventions at highest potential for cancer control.

10 projects were selected for funding.

1
Development of a Comprehensive Risk Prediction Model for BRCA1 and BRCA2 mutation carriers [TRANsIBCCS]

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The project aims to develop a comprehensive risk prediction model for BRCA1 and BRCA2 mutation carriers. The project is embedded in other international collaborations, which investigate either genetic or non-genetic risk factors. The unique feature of the TRANsIBCCS project is the combination of these and other risk factors in one study, which will enable the final development of the comprehensive risk prediction model and the prospective validation.

Women who carry a pathogenic mutation in the BRCA1 gene or BRCA2 gene have a high lifetime risk of breast and ovarian cancer. Because of this high risk, BRCA mutation carriers may need intensified screening, a risk reducing salpingo-oophorectomy or even a risk-reducing bilateral mastectomy. When in 2013 actress Angelina Jolie decided to have her both breasts prophylactically removed and reconstructed, this highly increased public awareness of this option for high risk women. As a result the uptake of risk reducing bilateral mastectomy increased, also in countries where this type of surgery was quite new. Still, the uptake is much higher in Northern than in Eastern and Southern European countries. Cultural and health care differences underlie this variation in uptake, but also uncertainty on the real breast and ovarian cancer risks. Indeed, risks vary between and within families, and therefore this project aims to develop a risk prediction tool that enables personalized risk prediction for BRCA1 and BRCA2 mutation carriers in the clinical genetic setting.
The researchers collaborating in the TRANsIBCCS project also collaborate in the prospective International BRCA1/2 Carrier Cohort Study (IBCCS) on reproductive and hormonal risk factors of breast and ovarian cancer and the cross-sectional study of the Consortium of Investigators of Modifiers of BRCA (CIMBA) on the associations of Single Nucleotid Polymorphisms (SNPs) and risks of breast and ovarian cancer. The TRANsIBCCS project has markedly increased the overlap between IBCCS and CIMBA, and added two important new components, which are strong risk factors of breast cancer apart from the BRCA mutation: pedigree-specific family history and breast density.

First, we chose BOADICEA (developed by A.C. Antoniou and D.F. Easton in Cambridge, UK) as the risk prediction model to be extended by the genetic and non-genetic risk factors. The model accommodates pedigree-based family history which is very important, because even among the BRCA1/2 mutation carriers family history proved to be strongly related with breast and ovarian cancer risks.

A relevant part of the family history effect on the risk of breast and ovarian cancer results from the location of the mutation and SNPs. The Polygenic Risk Score (PRS) combines the information of a large number of relevant SNPs. For the decision on which PRS will be used in the model for BRCA1/2 mutation carriers, we compared the results of CIMBA with those of the Breast Cancer Association Consortium (BCAC), which investigates SNPs in the general population. For BRCA1 mutation carriers the PRS for Estrogen Receptor negative breast cancer and for BRCA2 mutation carriers the general PRS for breast cancer, as developed in BCAC, were selected for the model. These PRS’s explained a similar part of the variances as the BRCA1- and BRCA2- specific PRS’s developed in CIMBA. So, for the PRS no BRCA-specific estimates will be needed in the extended BOADICEA model.

As the backbone of the BRCA part of the BOADICEA model we derived the mean absolute age-specific risk curves from prospective analyses of IBCCS. The mean cumulative breast cancer risk to age 80 years was 72% for BRCA1 and 69% for BRCA2 carriers, and the cumulative ovarian cancer risk 44% for BRCA1 and 17% for BRCA2 carriers. The range around these mean curves was strongly related with family history.

To determine whether the reproductive and hormonal risk factors needed BRCA-specific estimates in the risk prediction model, we conducted retrospective and prospective analyses in IBCCS. We investigated the effects of age at menarche, uptake of risk-reducing
oophorectomy, reproductive factors, use of oral contraceptives, body weight and height, smoking and use of alcohol and diagnostic radiation. Results of most risk factors were consistent with large meta-analyses in the general population, but the association between the first pregnancy and risk reducing salpingo-oophorectomy (BRCA1) and risk of breast cancer was discordant. So far, it seems that these important risk factors need a BRCA-specific estimate in the risk prediction model.

In many countries breast cancer screening of BRCA1/2 mutation carriers implies annual mammography and Magnetic Resonance Imaging (MRI). Recently, digital mammography has replaced the analogue imaging. We collected the various types of images and estimated breast density using STRATUS (developed by Dr. P. Hall, Stockholm, SE), a software program for automated readings. It proved to be quite challenging to derive a breast density estimate from the various images, made by various methods and devices. During the last part of the project we will investigate the magnitude of the association between breast density and breast cancer risk among the BRCA1/2 mutation carriers.

The TRANsIBCCS project enabled us to extend the IBCCS study group, to update the follow-up, to collect DNA samples, (follow-up) questionnaire data, breast images and pedigree information (co-funded by CR-UK) in each of the six countries. DNA samples were tested to assess the PRS (co-funded by Pink Ribbon). Breast density is currently being assessed using STRATUS. With all this information taken together, we will finalize the risk prediction model in retrospective analyses and validate the model prospectively. If the performance of the model proves to be adequate, the first version of the comprehensive risk prediction model can be expected during 2019. The new risk prediction model will support the difficult decision making of BRCA1/2 mutation carriers and their physicians on timing and uptake of preventive surgeries of breasts and ovaries.
Numerous cancers of the blood system can be cured by hematopoietic stem cell transplantation from healthy related or unrelated individuals (allo-HSCT). The curative effect relies on the ability of donor-derived immune cells to recognize tumour-specific antigens (TSAs), minor histocompatibility antigens (miHAg) and mismatched human leukocyte antigens (HLAs) on the patient's leukaemia and, upon engagement of these targets, to eliminate residual cancer cells. Unfortunately, a considerable proportion of transplanted patients still face disease recurrence, which has a severe prognosis and is a major cause of post-transplant mortality. This major unsolved medical issue warrants research on the largely unknown mechanisms that underlie resistance to the immune effects of allo-HSCT and post-transplantation relapses.

In previous studies, the two coordinators of the present project have observed that leukaemia relapses following allo-HSCT from HLA-mismatched donors are frequently due to the selective genomic loss of the incompatible HLA from the patient malignant cells ("HLA loss"). This renders leukaemia cells invisible to the circulating donor T cells and limits the effectiveness of therapeutic procedures commonly chosen to treat relapse, largely based on unleashing the breaks of circulating immune cells or on the infusion of lymphocytes freshly isolated from the donor2,3.
Despite these relevant clinical implications, the initial reports on HLA loss relapses failed to translate into major changes of clinical practice. This was due to the relatively small size of the single-center cohorts analyzed, insufficient to generate a general consensus, and to the lack of user-friendly laboratory tools apt to allow diagnosis of HLA loss in routine diagnostics.

In the HLALOSS project, six of the most active European HSCT centers (each involved in translational research and clinical activity) joined in a combined bench-to-bedside effort to develop and validate innovative techniques to detect HLA loss relapses, and employed these new cutting-edge tools to analyze an unprecedentedly large multicenter cohort.

Loss of mismatched HLA in leukemic cells after haploidentical HSCT. Schematic model of the causes and consequences of genomic loss of the patient-specific HLA haplotype at relapse after transplantation. Leukemic cells, heterozygous at diagnosis for the shared (in blue) and the mismatched patient-specific (in red) haplotype, are exposed to an intense immunological pressure after transplantation, mostly mediated by donor T cells expressing alloreactive T cell receptors (in green) and targeted against the mismatched HLA haplotype. This selective environment favors the emergence of mutant variants that lack the patient-specific HLA haplotype, and are therefore no longer recognized by donor T lymphocytes.

From: Horowitz et al, Bone Marrow Transplant. 2018 Apr 18
Novel Technologies

The joint effort of the two project coordinators recently lead to the development of an innovative assay (named “HLA-KMR”) based on quantitative polymerase chain reaction (qPCR), which allows the rapid and sensitive detection of HLA loss relapse variants. HLA-KMR assays target the most common HLA-A, -C, and -DPB1 allele groups, providing an informative marker to more than two thirds of the patients transplanted from partially HLA-incompatible related or unrelated donors. The assays require a relatively small amount of genomic DNA that can be recovered from peripheral blood or bone marrow specimens collected at the time of relapse without any need of further sample manipulation. Moreover, the assays require only standard laboratory instrumentation, available in the large majority of diagnostic facilities, and have a total turnaround time (from sample collection to result) of less than 48h. The technical performance of HLA-KMR assays has been extensively validated in three different laboratories on cell lines and patient samples, yielding remarkable reproducibility. Each of the 10 assays developed to date allows to unambiguously detect the presence of HLA loss leukemic variants with a sensitivity of up to 0.2%. HLA-KMR assays have transitioned with remarkable speed from research tools to diagnostic use, a process that was facilitated by their being made available as a commercial kit.

In parallel, an additional and synergistic methodology to detect HLA loss relapses was developed and validated through proficient collaboration with the DKMS Life Science Lab in Dresden. The proprietary Next Generation Sequencing (NGS) methodology in use at DKMS for HLA typing of candidate volunteer donors was adapted to the analysis of chimeric samples harvested at relapse after partially HLA-incompatible HSCT. This method is based on the locus-specific amplification and sequencing of six major HLAs (HLA-A, -B, -C, -DRB1, -DQB1, -DPB1), thus it is theoretically informative in all HLA-mismatched HSCTs. Moreover, by marking each sample with unique molecular identifiers, this method allows the simultaneous analysis of more than 40 samples per sequencing run, or more to 200 if only selected loci are sequenced. Through an ad hoc optimized bioinformatic pipeline of analysis, this NGS methodology allows to detect up to 0.5% of the sequences of interest in chimeric samples, with a negligible rate of false-positive results.

These two newly developed technologies not only cover an unmet medical need, but nicely complement each other in non-overlapping applications. HLA-KMR represents a fast and practical tool for any center that quickly needs to analyze a relapse sample to tailor clinical intervention. In contrast, the NGS-based methodology requires specific expertise and instrumentation, but has a significantly higher throughput and lower costs, representing the optimal solution for large retrospective studies such as the HLALoss project.
A Global Collaborative Study on the Immunobiology of Leukaemia Relapse

The six original partners from the HLALOSS consortium joined their efforts in collecting samples and detailed clinical and immunogenetic data from patients who relapsed after partially HLA-incompatible HSCT. Remarkably, after the initiative was presented at several international scientific meetings, other centres from around the globe became aware of the potential clinical relevance of the study and decided to join the consortium, which now sees involvement of a total of 20 centres from Europe (n=16), North America (n=3) and Asia (n=1). This unique transnational network has facilitated the collection of samples and data from more than 600 cases of hematologic relapse from adult patients who received allogeneic HSCT for a wide range of haematological malignancies. Of notice, this series did not only encompass relapses after HSCT from partially HLA-incompatible family donors, the setting in which HLA loss was initially described and more extensively investigated, but also a significant proportion of relapses after HSCT from unrelated donors, either adult volunteers or cryopreserved cord blood units, both settings in which the frequency and actual clinical relevance of HLA loss relapses is largely unknown. Relapse samples, and the relevant controls from patients and donors, are being analysed taking advantage of the new methodologies developed as part of the study. Although the study is still ongoing, approaching its final year, results are already of high interest, confirming the high frequency of HLA loss relapses after HSCT from related donors, but also highlighting that the relative risk of HLA loss can be predicted on the basis of the number and relative positioning (either in cis on the same chromosome or in trans on different chromosomes) of the HLA incompatibilities between donor and patient. If confirmed, these findings may rapidly translate into new algorithms for donor selection, and into new indications for the post-transplantation follow-up of patients, including tighter monitoring and prophylactic approaches for patients deemed at risk of experiencing HLA loss relapses.

3
Cancer prevention through improved familial risk assessment and gene discovery [FAMILIAL CANCER]

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The project had two complementary work-packages (WPs). WP1 focused on familial risk and on clinically useful risk prediction tools. In WP2, the aim has been to identify novel genes that predispose to familial cancer. We followed the empirical gene discovery paradigm by identifying high-risk families diagnosed with the same cancer in several family members in at least 3 generations. Such families and samples were identified mainly from the world's largest biobank of familial cancers in Szczecin and some from the medical genetic clinic in Groningen. Whole-genome sequenced (WGS) has been completed at the DKFZ core facility for close to 100 families with 300 individuals, including patients with cancer and healthy family members. Main focus has been on colorectal cancer. An important product has been the development of a germline sequencing pipeline applicable to any pedigree-based WGS data.

As of June 2018 some 15 papers have appeared based on the Transcan funding. Some examples are discussed below.

WP1. Relatives of cancer patients are at an increased risk of the same (concordant) cancer, but whether they are at a risk for different (discordant) cancers is largely unknown – beyond well characterized hereditary cancer syndromes - but would be of major scientific and clinical interest. We therefore decided to resolve the issue by analysing familial risks when family members were diagnosed with any discordant cancers. We compared the population impact of concordant to discordant familial cancer. The Swedish Family-Cancer Database (FCD) was used to calculate familial relative risks (RRs) for family members of cancer patients, for the 27 most common cancers. Population attributable fractions (PAFs) were estimated for concordant and discordant family histories. Discordant cancers in the family were detected as significant risk factors for the majority of cancers, although the corresponding RRs were modest compared to RRs for concordant cancers. Risks increased with the number of affected family members with the highest RRs for pancreatic (2.31),...
lung (1.69), kidney (1.98), nervous system (1.79) and thyroid cancers (3.28), when 5 or more
family members were diagnosed with discordant cancers. For most cancers, the PAF for
discordant family history exceeded that for concordant family history. Our findings suggest
that there is an unspecific genetic predisposition to cancer with clinical consequences. We
consider it unlikely that shared environmental risk factors could essentially contribute to
the risks for diverse discordant cancers, which are likely driven by genetic predisposition.
The identification of genes that moderately increase the risk for many cancers will be a
challenge. This paper was published by Frank et al, Int J Cancer 2017, and how discordant
risks influence prostate cancer and melanoma were published by the same authors in
European Urol and Scientific Reports also in 2017.

WP2. In the course of our WGS efforts, we developed a pipeline for analysing germline
genomes from Mendelian types of pedigrees. The variant calling step distinguishes three
types of genomic variants: single nucleotide variants (SNVs), indels and copy number
variants (CNVs), which undergo technical quality control. Mendelian types of variants are
assumed to be rare and usually variants with frequencies higher that 0.1% are screened out.
Segregation in the pedigree allows variants to be present in affected family members and
not in old unaffected ones. The effectiveness of variant segregation depends on the number
and relatedness of the family members; if over 5 third-degree (or more distant) relatives are
available the experience has shown that the number of likely variants is reduced from many
thousands to a few tens. These are then subjected to bioinformatic analysis, starting with
the combined annotation dependent depletion (CADD) tool, which predicts the likelihood
of the variant being deleterious. Different sets of tools are used for coding variants, 5’-
and 3’-UTRs, promoter/enhancer areas and intergenic variants. The likelihood of success of
the present genomic pipeline in finding novel high- or medium-penetrant genes depends
on many steps but first and foremost, the pedigree needs to reasonably large and the
assignments and diagnoses among the members need to be correct. The first version of
this pipeline was published by Försti et al, Hereditary cancer in clinical practice 2016, and
the second version is in press, Kumar et al, Sci Reports 2018.

The TRANSCAN funding has been an important instrument to facilitate European
collaboration. It has been covering in a timely fashion the gap between national funding,
which does not normally cover foreign participation, and the HORIZON 2020 types of
megaprojects. It was also appreciated that the focus was clear and the mega bureaucracy
typical of the megaprojects was avoided. In our case, collaboration between the partners
will continue and the first project meeting beyond the TRANSCAN funding has already been
decided.