THE ERA-NET: ALIGNING NATIONAL/REGIONAL TRANSLATIONAL CANCER RESEARCH PROGRAMMES AND ACTIVITIES

THE SECOND TRANSCAN-2 SYMPOSIUM

September 2017 in Madrid, Spain

TRANSCAN-2 organizes scientific symposia for researchers, with projects funded through TRANSCAN and TRANSCAN-2 JTCs, and for ministries and agencies, funding research and innovation, from EU and beyond, which are collaborating in TRANSCAN-2.

The symposia aim to encourage the exchange of information and to increase the awareness of the outcome and the impact of the projects.

The Second Symposium is focused on projects from TRANSCAN JTC 2012 and 2013, which will be presented to invited experts from the field of cancer prevention, whilst also promoting young scientists from the funded consortia, through poster presentations and networking activities.

NEWS ON THE FIRST TRANSCAN JOINT TRANSNATIONAL CALL 2011:

"Validation of biomarkers for personalised cancer medicine"

SHORT INFO ON JTC 2011:

- Participation of 15 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 oncology, oriented towards a rapid application of new and more selective and effective tools and strategies for the prevention, diagnosis, early detection, and therapy of neoplastic diseases. Proposals had to cover at least one of the following areas: prevention; early detection; diagnosis; prediction of response or resistance to treatment; prediction of treatment toxicity.
- 10 projects were selected for funding.
FIRSTHAND SUCCESS STORY FROM THE JTC 2011:

PROVABES: PROspective VAilidation of Biomarkers in Ewing Sarcoma for personalised translational medicine

Project Coordinator
Uta DIRKSEN: University Hospital Muenster, Germany

The PROVABES story by Uta Dirksen

The primary aim of PROVABES is a systematic validation of selected biomarkers in Ewing sarcoma (EwS) in a prospective joint investigational program. The main focus of our consortium lies on the clinical validation of biomarker sensitivity and specificity. To meet this goal, a highly selected number of biomarkers, i.e., chromosome (chr) 1q gain, chr p16 alterations, hsamir 34a, MGST1, LGALS3BP, STEAP1 and DKK2 is assessed in biomaterials of patients treated within the GCP-compliant European clinical trials that are analysed and will be published in accordance with the “REMARK” recommendations. A secondary focus is the exploratory analysis of the Ewing sarcoma genome, epigenome and on the detection and validation of liquid biopsy markers. Main source of samples was the Phase III randomised international multicentre trial EWING 2008 (EudraCT 2008-003658, NCT00987636). Standard operation procedures have been discussed and agreed among partners, Tools for biomaterial collection (tumourbox) and ready to use instructions for the participating centres have been implemented. Collection and distribution of biomaterials has been initiated and coordinated. Prospectively collected biomaterial and biomaterial for assay validation has been distributed to partners.

Whole genome analysis
Our consortium described the genomic landscape of Ewing sarcoma, and showed that Ewing sarcoma has a very low mutational rate (0.15/megabase). The low mutational rate is in concordance with other translocation- positive malignancies in young people. In more than 10% of patients, somatic mutations in STAG2, that encodes a subunit of the cohesin complex that regulates sister chromatid
exchange during mitosis and meiosis. Other genetic alterations include deletion of CDKN2A and mutations in TP53. In a retrospective study we found hints of a prognostic relevance of combined STAG 2 and TP53 mutations. Furthermore, we could demonstrate the relevance of mutations in FGFR1. (1, 2)

**Copy number alterations (CNA)**

At current stage of analyses, 9.0% patients (pts) had 1q gains, and 9.4% pts had 16q losses. While no significant correlation with histological response was found for 1q gains (P>.10), more pts with 16q losses had a poor histological response (50%) compared to pts with no 16q losses (19.8%) (P=.03). The effect was triggered by a low number of patients with 16q losses, but remained stable in binary logistic regression corrected for age, sex, tumour volume and tumour site (OR= 4.16; 95 CI 1.18-14.65; P=.03). Pts with 1q gains had a lower OS in univariate Cox regression (OR=2.82; 95 CI 1.04-7.66; P=.04) that remained stable in multivariable analyses (OR=2.36; 95 CI 0.87-6.43; P=.09). Pts with 16q losses had a lower OS in univariate Cox regression (OR=3.70; 95 CI 1.45-9.45; P<.01) that remained stable in multivariable analyses (OR=3.36; 95 CI 1.30-8.69; P=.01). The chromosome 1q loss and 16q loss are associated with a lower overall survival in patients with localized disease. These markers may represent prognostic markers in Ewing sarcoma. The chromosome 16q loss is associated with a poor histological response and may represent a predictive marker in Ewing sarcoma.

**Proteom**

For our analyses, samples from patients (pts) with localized disease were analysed. The majority of patients were recruited from the EWING 2008 trial (78.4%); 35.4 were male and 45.2% were female. At current stage of analyses, 82.9% pts had STEAP expression. DKK was detected on 66.7% of the tumour samples and 89.7% showed an EZH2 immunoreactivity reaction. No significant correlation with histological response was found for STEAP (3), DKK2 and EZH2 biomarkers in univariate correlations and binary logistic regressions analyses (P>.10). For STEAP and EZH2, no significant differences were found in event-free (EFS) and overall survival (OS) in univariate log-rank tests and multivariable Cox regression analyses, including other prognostic factors like age, sex, tumour volume, and tumour site. For DKK2, a better survival (OS) was found for positive samples in univariate analyses (P=.04; EFS: P=.07). The markers were tested in a score that defines the level of expression. We are currently investigating the impact of very high levels of expression versus no expression.

**Epigenome**

We analysed the epigenetic pattern in Ewing Sarcoma by using a novel bioinformatic methods. A Ewing-specific epigenetic signature was identified and this finding implicates that enhancer-reprogramming may be a specific molecular phenotype in Ewing sarcoma. There was an inter-individual heterogeneity which is in sharp contrast to the low number of genetic alterations (see WP1). Our data highlight the potential importance of epigenetic heterogeneity as a major contributor to the observed phenotypic heterogeneity among genetically similar cancers. The epigenetic pattern and seems to determine the clinical outcome in Ewing sarcoma patients and may therefore represent a prognostic factor. This needs to be analysed in upcoming prospective studies. (4)

**Liquid biopsies**

We pioneered a method that allows detection of the informative genomic fusion sequence from a minimal amount of DNA, which has been validated in EwS xenograft mouse models. In the worldwide fist pilot study, 234 serial blood samples of 20 EwS patients were analysed and correlated to the tumour burden assessed by imaging studies prior and during multimodal treatment. The
majority of patients displayed reduction of ctDNA after induction-chemotherapy. Recurrence of increasing ctDNA levels indicated relapse, often ahead of clinical manifestation. Pre-analytical processes were harmonized by standard operation procedure (SOPs) developed within PROVABES and advanced methodology for fusion site sequencing has been developed to facilitate efficient assay design. Limitations, including the inability to distinguish residual tumour from non-tumour residual tissue masses and significant challenges for assessing response to cytostatic agents when tumour cell death and reduction in tumour mass is not expected. We expect that with our method we will be able to track tumour response parallel to the active anti-cancer treatment. (5)

**Project consortium**
Stefan BURDACH, Kinderklinik, Germany
Enrique DE ALAVA, University Hospital of Salamanca, Spain
Olivier DELATTRE, Institut Curie, France
Heinrich KOVAR, St. Anna Kinderekbsforschung e.V, Children’s Cancer Research Institute, Austria
Piero PICCI (Italy) Istituto Ortopedico Rizzoli, Italy
Sue Ann BURCHILL, St James’s University Hospital, United Kingdom

**Selected references**

This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under Grant Agreement No 643638.