Co-funded by the Horizon 2020 programme of the European Union

"ThruBlood"

Clinical validation of Trop-2 as a serum biomarker for monitoring of disease-course in patients affected by breast, ovary and colon cancer

Project PIC 944224288

Summary

Cancer is the leading cause of death globally. Current follow-up approaches adopted for the screening of patients in the post-treatment phase of breast/colorectal cancer rely on technologies that are affected by strong limitations in terms of cost-effectiveness (i.e. imaging technologies) and performance (e.g. serum biomarkers like HER2 for breast, CA-125 for ovary and CEA for colon).

Moreover, the drivers of disease recurrence and metastases are markedly heterogeneous and often depend on features of primary cancer such as biological aggressiveness, response to therapy and prognostic features. As a result, the set-up of effective therapeutic strategies for individual patients is remarkably challenging. This reduces the efficacy of administered therapies, is at risk of considerably reducing the quality of life of the patient and poses a heavy management and financial burden on the health system. To achieve the goal of effective choice among effective therapies, novel cancer biomarkers with high specificity and sensitivity are urgently needed. Such biomarkers will allow a timely and accurate monitoring of the disease course of patients bearing, e.g. breast or colorectal cancer that have been diagnosed and treated.

After over 10 years of research, the staff of Oncoxx Biotech, a Cancer Biotech Company, has discovered in cancer patients and pre-clinical models that **Trop-2 is the only gene consistently expressed at high levels** in the majority of cancer cells that generate metastatic tumours in vivo. With this information at hand, we started our studies to determine whether Trop-2 also is a circulating, prognostic marker of tumors. We found that this protein is consistently released in the bloodstream, when a subject is developing a relapse from most of the analyzed cancers. This is opening a completely new pathway for introducing a **low-cost and widely available serum biomarker** for monitoring the disease progression.

Within this R&D we have created **three monoclonal proprietary antibodies anti-Trop-2,** as a possible **quasi universal cancer target**. The antibodies differentially inhibit growth of (i) primary tumors and (ii) metastatic cells in pre-clinical models and synergize in vivo.

The top two biomarker's features are **high specificity (100%)** and **high sensitivity (80%),** that coupled with a **low-cost**, will enable the effective implementation of the **novel high throughput** and **high accuracy assay** through the most common diagnostic procedures, like the ELISA test.

This is a sound starting point, which positions **Trop-2 beyond the biomarkers currently adopted**.

We, at Oncoxx Biotech believe that our studies on the Trop-2 protein have paved the way for the introduction of a cancer serum biomarker with unprecedented performance. Our plans are to clinically validate such biomarker in independent, extended cohorts of patients with breast, colon, ovarian cancers.

Study design: measurement of Trop-2 level in blood serum of cancer patients at surgery, 1 month after surgery and at 6 months intervals afterward. Trop-2 detection will be performed by a newly developed, state-of-the-art ELISA assay. Detection and levels of circulating Trop-2 will be correlated with clinical and pathological status. Five leading medical institutions will be involved from Italy, Germany, France and Croatia, to achieve high enrolment capacity, medical impact and ultimate success of the project.

Strategic Objective 1 of the study will be the Assay Validation: an innovative ELISA assay will be developed, with high specificity and sensitivity, coupled with a low-cost, high-throughput assay for effective implementation within a diagnostic procedure.

Strategic Objective 2 will be Laboratory Validation: To validate procedures for measuring circulating Trop-2 in clinical settings in terms of (i) accuracy, (ii) reliability, (iii) predictive power, (iv) speed, (v) cost efficiency, to pave the way for extensive, routine use in cancer centers worldwide.

Strategic Objective 3 will be Clinical Validation: (a) To validate Trop-2 as a disease history predictor, through systematic measurement of Trop-2 in the serum, across large groups of breast, ovarian and colon cancer patients, at diagnosis and after surgery. Such biomarker will allow: (i) the early detection of patients with aggressive tumours, (ii) the assessment of the tumour burden at any given stage of the disease, (iii) to monitor tumour-bearing patients to draft a relapse-risk profile.

Final Objective of the study will be the Clinical Validation of the relationship of circulating Trop-2 levels with the biological characteristics of the tumor, to allow the best available therapy and the best monitoring of disease course.

Our ambition is to **revolutionize the way disease-course monitoring and treatment** are conceived, allowing clinical laboratories to avail a reliable diagnostic tool capable to accurately identify those patients at high risk of developing metastatic relapse.

The main outcomes of such actions are summarized as follows:

1. We have designed and finalised the project work plan whereby AJInnuscreen, Berlin, Germany will participate in the development of the final ThruBlood assay.

2. We have defined the final objectives of the Clinical Validation to be possibly carried out under SME Instrument Phase 2 project, taking into account both the starting considerations and the additional expertise and background coming from the participants involved in the WP.

3. We have designed and finalised the project work plan for the clinical validation and the allocation of responsibilities amongst the participants, which will be CIRM, Milano, Italy for the organisation and management of the clinical validation.

4. We have analysed the business opportunity for the ThruBlood product, given the current unmet needs and the market drivers that are responsible for the cancer biomarker market growth. A first agreement has been reached with AJInnuscreen, Berlin, Germany for carrying out a Phase 3 of the project for market entry and commercialisation of the final ThruBlood assay.

As a key example, the global market for Predictive Breast Cancer diagnostic and drug technologies was valued at $23.4 billion by a recent report, twice as much as that of the drug market, and is expected to rise at a compound annual growth rate (CAGR) of 2.5%, to reach $24 billion by the end of 2016. Perspectives are to have the potential to acquire a significant fraction of this market in regard to cancer diagnostics.

Relevant publications, patents and licenses

1. Guerra E., Trerotola M., Dell' Arciprete R., Bonasera V., Palombo B., El-Sewedy T., Ciccimarra T., Crescenzi C., Lorenzini F., Rossi C., Vacca G., Lattanzio R., Piantelli M. and Alberti S. A bi-cistronic Cyclin D1-TROP2 mRNA chimera demonstrates a novel oncogenic mechanism in human tumors. Cancer Res. 68: (19): 8113-8121 (2008).

2. Plebani R., Oliver G.R., Trerotola M., Guerra E., Cantanelli P., Emerson A., Harkin P.D., Kennedy R.D. and Alberti S. Long-range transcriptome sequencing reveals cancer cell growth regulatory chimeric mRNA. Neoplasia 14 (11): 1087-1096 (2012).

3. Trerotola M., Cantanelli P., Guerra E., Tripaldi R., Aloisi A.L., Bonasera V., Lattanzio R., de Lange R., Weidle U.H., Piantelli M. and Alberti S. Up-regulation of Trop-2 quantitatively stimulates human cancer growth. Oncogene 32(2):222-33 (2013). Epub 2012 Feb 20.

4. Ambrogi F., Fornili M., Boracchi P., Trerotola M., Relli V., Simeone P., La Sorda R., Lattanzio R., Querzoli P., Pedriali M., Piantelli M., Biganzoli E. and Alberti S. Trop-2 is a determinant of breast cancer survival. PLoS One 9 (5): 1-11 (2014).

5. Guerra E., Trerotola M., Tripaldi R., Aloisi A.L., Simeone P., Sacchetti A., Relli V., D' Amore A., La Sorda R., Lattanzio R., Piantelli M. and Alberti S. Trop-2 induces tumor growth through Akt and determines sensitivity to Akt inhibitors. Clin. Cancer Res. 22 (16) 4197-4205 (2016).

Patents

PCT: S. Alberti and Emanuela Guerra "Use of Trop-2 as predictive marker of response to anti-tumor therapy based on inhibitors of CD9, Akt and molecules of the tetraspanin signalling network" - PCTIIT2013/000139 – applied for on the 16th May 2013.

Patent: S. Alberti "Oligonucleotide sequences that inhibit the expression of chimeric mRNA that control the growth of tumor cells and their use in the medical field" - applied for in Chieti on the 12th Nov. 2012. SIMBA N° CH2012A000016.

PCT: S. Alberti and Emanuela Guerra "Oligonucleotide sequences able to silence the expression of the CYCLIN D1/TROP2 chimera and use thereof in the medical field" - PCTIIT2009/000437 – applied for on the 25th Sep. 2009. Granted on the 13th Aug. 2013 – US8507664B2.

PCT: S. Alberti "Anti-Trop-2 monoclonal antibodies and uses thereof in the treatment and diagnosis of tumors" – PCT/IT2009/000035 – applied for on the 5th Feb. 2009. Granted on 2014.

PCT/EP: A. Anastasi, F. Petronzelli, S. Alberti, R. De Santis "Anti-EpCAM antibody and uses thereof" (548/PCT/EPBS000R548) – applied for on the 2nd Apr. 2008; approved on the 15th june 2011 #2142570; 548-PCT-US, approved on the 28th Nov. 2012 as US8318911B2.

Patent: S. Alberti "New technologies for the analysis of the proteome" – applied for in Chieti (Application number CH05A000007) on the 14th Apr. 2005; approved on the 10th jul. 2009 - N° 0001363767. Patent: S. Alberti and A. Sese "Nucleic Acids Sensors" – applied for in Chieti (Italy) (Application number CH02A000009) on the 16th Sep. 2002; approved on the 23rd feb. 2006 - N° 0001332234.

Licenses

1997: S. Alberti pRK-5-C-GFP and the pRK-5-N-GFP mammalian expression vectors, to BD Biosciences Pharmingen, San Diego, USA.

2013: S. Alberti, Oncoxx Biotech, 2EF and 2G10 monoclonal anti-Trop-2 antibodies, to Millipore-Merck, USA.