Tumor Infiltrating Myeloid Cell Compartment

In the western world about 30% of the population will develop cancer once in their lifetime and this frequency is increasing. Immunity plays a central role in tumorigenesis. The **t**umor **i**nfiltrating **m**yeloid **c**ell **c**ompartment (timcc) of the innate immune system can either prevent or contribute to cancer. The mechanisms underlying pro- versus anti-tumor programming of the tumor microenvironment remain largely obscure. The ITN-TIMCC provides substantive and methodological training in the functional analysis of the timcc and aims to gain insight into the molecular and cellular pathways underlying the pro versus anti-tumor programming of the timcc resulting in the identification of new therapeutic targets to enhance the tumor antagonizing activity and to suppress the tumor promoting activity of the timcc.

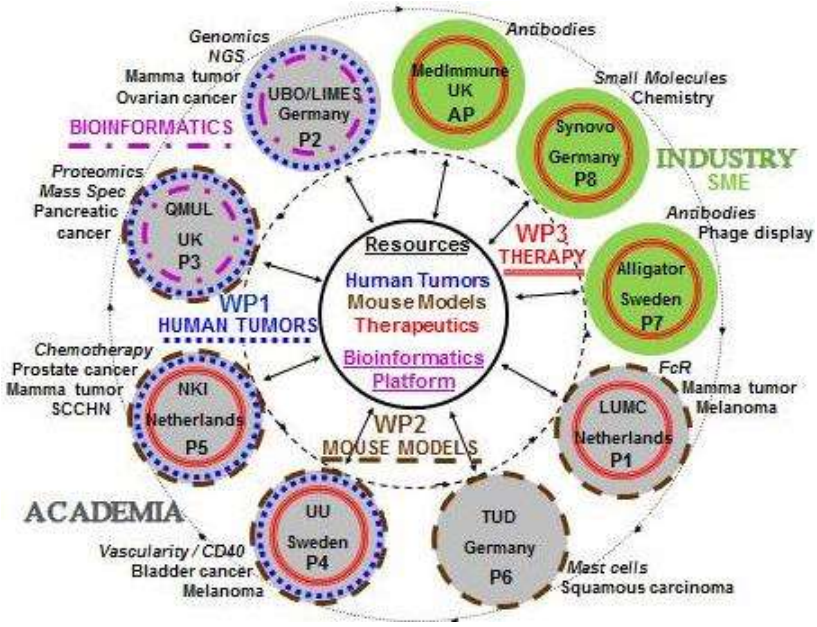
METHODOLOGICAL APPROACH

Building a training and research network

- A platform for genomics, proteomics and bioinformatics analysis and shared resources of human tumor biopsies, pre-clinical mouse tumor models and therapeutics.
- A 'circle' of 6 academic research groups (P1-6) with a large variety of expertise in *tumor immunology,* 2 SME partners (P7,8) developing therapeutics interfering with the immune system and 1 associated private sector partner (AP) providing additional training.

Focussing on:

- Analysis of the myeloid cell compartment in human biopsies using different technical approaches e.g. RNAseq, proteomics, immune-histochemistry, Flow cytometry combined with bioinformatic analysis.
- Establishing the corresponding models of a variety of human tumors in genetically modified mouse strains.
- Studying in the experimental mouse models the tumor infiltrating myeloid cell compartment in tumorigenesis and during therapeutic intervention.



Expected final results, their impact and use

Immunotherapy is booming. A recent avalanche of publications reported promising results of pre-clinical and clinical studies with agonistic antibodies (e.g anti-CD40), check point blocking antibodies (e.g. anti-Pd1, anti-Pd-L1, anti-CTLA4), cell depleting antibodies (anti-CTLA4) and small molecules (CSF-1R inhibitor) interfering with immune mechanisms. The timcc has a strong impact on the outcome of immunotherapy. It is generally believed that an effective immunotherapy requires the selective depletion and/or reprogramming of the tumor microenvironment to optimize the tumor antagonizing activity of the immune system. The TIMCC training and research program will gather knowledge and skills to develop, in the near future, new therapeutic strategies on one hand to promote the tumor antagonistic properties of the timcc and on the other hand to suppress its tumor-promoting properties. These strategies will become an intrinsic part of future successful immunotherapy.

TIMCC activities are organized through 6 work packages:

WP1-3: Research

WP1: Genomic characterization, proteomics and bioinformatics on the myeloid cells compartment in human tumor biopsies

WPP2: The tumor infiltrating myeloid cell compartment in experimental mouse tumor models

WP3: The tumor infiltrating myeloid cell compartment in therapy

WP4: Training

WP5: Dissemination, Outreach and IP

WP6: Management

Short term objectives - studying the following aspects of timcc:

- Diversity and function of tumor associated macrophages
- Role of mast cells in tumor development
- Significance of myeloid cells and their mediators in chemotherapy responsiveness of cancer
- FcR and complement mediated activation of myeloid cells by tumor specific antibodies
- The interplay between endothelial cells and myeloid cells in the tumor microenvironment
- Immunomodulatory therapeutic antibodies targeting the tumor microenvironment
- Small molecules interfering with effector pathways of timcc

DISSEMINATION RESULTS

Publications

Cancer Immunol Res (Sandin et al, 2, 2014); OncoImmunol (Sandin et al, 3, 2014); Immunity (Xue et al, 40, 2014); J Immunother (Liljenfeld et al, 37, 2014); Front Immunol (Schmidt et al, 5, 2014); Nucl Acid Res (Krebs et al, 42, 2014); FASEB J (Huang et al, 2, 2015)

Patent Applications

US Patent: Combinations of microenvironment modulators and anti-tumor antibodies

US Patent: R-isomer of RO320195

RESEARCH ACTIVITIES AND RESULTS (WP1-3)

We established an *in vitro* model system of human monocyte-derived TAM used to examine their transcriptional and epigenomic signature. In patients treated with chemotherapy we found deviations in cell numbers of monocytes (Mo) suggesting an influence of chemotherapy on Mo activation. As a model for macrophage (Mf) infiltration into the tumor infiltrating microenvironment we have established Mo-derived Mf from human buffy coats that are further differentiated into Mo (IL-4) and Mo (IFNg) cells. We produced the largest dataset for human macrophage activation published so far and contributed to a proposal for new nomenclature of macrophage activation. In order to characterize TAM in prostate cancer and squamous cell carcinoma of the head and neck biopsies we have optimized the procedure to obtain $CD45^+$ $CD14^+$ $CD163^+$ TAM. Transcriptome analysis by RNA-sequencing of Mf from different organs and breast cancer in a mouse tumor model suggests a specific transcription factor program in TAMs versus all other Mf isolated. In breast cancer patients we have phenotypically characterized different Mo populations in blood since these cells are potential precursors for TAMs. We have established a change in frequency of different Mo populations after certain therapy regimens with an increase of inflammatory Mo suggesting an influence of therapy on these immune cells. We established a 3D mouse pancreatic cancer to study macrophage/tumor cell interactions and measured the migration of myeloid and other cells. This model enables us to study how the tumor stroma, and matrix stiffness, modulates the infiltration and activation of T cells and recruitment of myeloid cells. We found that pancreatic ductal adenocarcinoma (PDAC) cell lines respond to CX3CL1 stimulation with induction of actin polymerization. This may suggest that tumor cells can migrate to CX3CL1 gradients towards neurons. Unexpectedly, we found, using a novel more accurate mast cell deficient mouse model, that tumourigenesis in squamous carcinoma was not affected by the absence of mast cells, indicating that, in conflict with the literature, mast cells are not required in this model. For further studies we generated a transgenic mouse that allows inducible expression of oncogenes in epidermis. In a spontaneous mouse mamma tumor model we found that Mf ablation positively influenced cisplatin response whereas ablation of neutrophils did not. Mf blockade combined with cisplatin triggers a rewiring of the inflammatory tumor microenvironment resulting in therapy resistance. These data highlight the importance for optimally matching chemotherapeutics with immunomodulatory compounds. Combination of CD40-activating treatment with anti-angiogenic therapy was evaluated in mouse tumors showing enhanced anti-tumor effect and decreased recruitment of Myeloid Derived Suppressor Cells. We started to study anti-HER2/neu/ anti-CD40 combination therapy in the spontaneous neuT mammary carcinoma model. We found that treatment of Melanoma with TA99 antibody combined with gp100 long peptide vaccination increased survival of WT mice but not FcgR KO mice indicating that the therapy is FcgR dependent. To study the mechanism in more detail we have developed a series a novel conditional FcgR KO mouse strains. We examined the anti-tumor effects and ramifications on the tumor-infiltrating myeloid cells following administration of anti-CD40 in mouse tumors. We found that while anti-CD40 decreases the number of infiltrating myeloid cells in certain tumor models, it does not directly correlate with its efficacy. We have established an *in vitro* assay employing co-incubation of cancer cell lines and bone marrow-derived macrophages with tumor-conditioned media for screening compounds for effects on IL12, TNFα, NO and M1/M2 Mf phenotypes.

TRAINING

A Training & Education Platform was established under supervision of the TIMCC training officer (P6-TUD), organizing progress report meetings (8 Oct 2013, UK; 4 Feb 2014, NL; 21 May 2014, SE; and 23 Oct 2014, DE) or videoconferences. The following Training workshops have been organised *'Analysis of Human Tumor Biopsies'* 9 Oct 2013 (P3-UK), '*Angiogenesis*' 22 May 2014 (P4-SE), Chair: Dr. Lena Claesson-Welsh; *'Genomics Analysis Using Open Source Tools'* 24 Oct 2014 (P2-DE). A Masterclass on *'Inflammation and Cancer'* was organized 4-5 Feb 2014 (P5-NL), Visiting Scientist: Dr. Toby Lawrence (Center of Immunology, Marseille, FR); and two seminars on Careers in Industry were presented by Michael Burnet (P8-Synovo). Two scientific Symposia have been organized: *'TIMCC in Human Tumours',* on 7 Oct 2013 (P3-UK), with Visiting Scientist: Dr. Olivera Finn (Pittsburgh, US), and with about 100 participants from outside the network; and *'Systems Approaches meet the Tumor Microenvironment'* on 22 Oct 2014 (P2-DE), with keynote speaker Dr. Peter Murray (St. Jude Children's Hopsital, US) with about 50 participants from outside the network.

PROJECT COORDINATOR

Dr. Sjef Verbeek
Associate Professor
Dept. of Human Genetics
Leiden University Medical Center
Albinusdreef 2
2333 ZA Leiden (NL)

E-mail: j.s.verbeek@lumc.nl
Website: www.timcc.eu
Twitter: https://twitter.com/@TIMCCFP7

TIMCC PARTNERS

1. Leiden University Medical Center (NL)
   Sjef Verbeek, coordinator and PI
   Babs Teng, project manager
2. University of Bonn (DE)
   Joachim Schultze, PI
3. Queen Mary University of London (UK)
   Frances Balkwill, PI
4. University of Uppsala (SE)
   Anna Dimberg, Sara Mangsbo, PIs
5. Netherlands Cancer Institute (NL)
   Karin de Visser, Jan Paul de Boer, André Bergman, PI's
6. Technical University Dresden (DE)
   Axel Roers, PI
7. Alligator Bioscience AB (SE) - SME
   Peter Ellmark, PI
8. Synovo GmbH (DE) - SME
   Michael Burnet, PI
9. MedImunne (UK) – Associated Partner
   Viia Valge-Archer, PI

▼ = partner ▼ = SME ▼ = Associated Partner

MANAGEMENT

The TIMCC project is funded with € 3.4 million through the EU 7th framework program. A supervisory board consisting of all PIs meets at yearly management meetings: Kick-off, 9 Jan 2013 (NL), 1st Annual Meeting, 8 Oct 2013 (UK); Mid Term Meeting, 22 Oct 2014 (DE) and regular web-based meetings.

RECRUITMENT

12 ESRs and 2 ERs were recruited, 10 female and 4 male, 10 from EU Member States, 2 from EU Candidate states and 2 from Third Countries.