Journal for ImmunoTherapy of Cancer

Volume 2
Supplement 2
March 2014

Proceedings book of the 1st Immunotherapy of Cancer Conference (ITOC1)

Munich, Germany
12-14 March 2014

Meeting abstracts and Programme

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The Supplement Editors declare that they have no competing interests.

Publication of this supplement was funded by the Biotherapy Development Association, a non-profit organisation.

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J Immunother Cancert-2-S2-prelims.indd 1 13/02/2014 11:59:21

Journal for ImmunoTherapy of Cancer

Volume 2
Supplement 2
March 2014

Journal information

Journal for ImmunoTherapy of Cancer (ISSN 2051-1426) is published online by:

BioMed Central Ltd
Floor 6, 236 Gray's Inn Road
London WC1X 8HB
United Kingdom
T:+44 (0) 20 3192 2000
F: +44 (0) 20 3192 2010
E: info@biomedcentral.com
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The journal can be found on the web at the following address:
http://www.immunotherapyofcancer.org/

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CONFERENCE PROGRAMME

WEDNESDAY 12 MARCH 2014

11:00-12:30 Bristol Myers Squibb Satellite Symposium

Opening plenary session – Chairs: H Zwierzina (Austria) and V Nussler (Germany)

13:00 Welcome address
H Zwierzina (Austria)

13:10 S1. KEYNOTE LECTURE: Cancer immunotherapy - current and future advancements*
A M M Eggermont (France)

13:40 Combinatorial immunotherapy and anti-angiogenic approaches in ovarian cancer*
L Kandalaft (USA)

14:00 S3. Toll like receptor 7 agonists for cancer immunotherapy*
S Endres (Germany)

14:20 S4. Molecular pathways associated with immune modulation and thalidomide analogues*
R Chopra (USA)

14:40 S5. Proffered paper: Maintenance therapy of metastatic colorectal carcinoma with the TLR-9 agonist MGN1703: clinical and immunological predictive pretreatment factors of activity in the IMPACT trial
H J Schmoll (Germany)

14:50 S6. Proffered paper: Anti-tumour immunomodulatory activity of heat shock protein Hsp70 in therapy of malignant brain tumours: preclinical and clinical data
M Shevtsov (Russian Federation)

15:00-15:30 Coffee break

Plenary session: Cancer immunology – Chairs: T Gajewski (USA) and P Romero (Switzerland)

15:30 S7. KEYNOTE LECTURE: Immunobiological properties of the human cancer stem cells
C Maccalli (Italy)

16:00 S8. Bavituximab: a novel phosphatidylserine-targeting immunotherapy for the treatment of cancer*
J Shan (USA)

16:20 Targeting the myeloma microenvironment with immunotherapy*
Speaker to be announced

16:40 BiTe antibodies: from ex vivo models to clinical application in solid tumours*
P Kufer (Germany)

17:00 S9. Proffered paper: Identification of novel immune checkpoints as targets for cancer immunotherapy
G Cojocaru (Israel)

17:15-18:45 Poster session: Monitoring of immunotherapy

P10. Concomitant gemcitabine therapy negatively affects DC vaccine-induced CD8+ T cell and B cell responses but improves clinical efficacy in a murine pancreatic carcinoma model
C Bauer (Germany)

P11. Developing an immunotherapy strategy for the effective treatment of patients with non small cell lung cancer (NSCLC): strategies to evaluate immunity in patients on clinical trials
M Neuberger (Germany)

* Abstract not submitted for publication

P12. Combination of radiotherapy and chemotherapy with dendritic cell immunotherapy in glioblastoma patients induces NK and NKT cell responses
S Pellegatta (Italy)

P13. Intra-tumoral and surrogate immune responses in patients treated with the engineered anti-PD-L1 antibody (MPDL3280A)
M Kowanetz (USA)

17:15-18:45 Poster session: Immunomodulation

P14. Cell-based gene delivery leverages conventional immunotherapy for cancer
C Günther (Germany)

P15. A genetic mouse model to identify the role of the immune adapter protein MyD88 in colorectal cancer
A Holtorf (Germany)

P16. Differential susceptibility of human and mouse NK cells to malignant cell-induced abnormalities in autologous combinations: a potential mechanism for the NK cell-based immunotherapy efficacy
G Sconocchia (Italy)

P17. RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells
M Schnurr (Germany)

P18. The tumour leukocyte infiltrate is the key predictor for therapeutic response to catumaxomab therapy
B Mayer (Germany)

P19. Immunmodulation of blasts in AML-Patients (pts) with clinically approved response modifiers to improve antileukaemic T-cell reactivity: an ex vivo simulation of the clinical situation
H Schmetzer (Germany)

P20. Lack of T cell exhaustion in acute myeloid leukaemia
F Schnorfeil (Germany)

P21. NK-cell dysfunction in human renal carcinoma reveals diacylglycerol kinase as key regulator and target for therapeutic intervention
P Prinz (Germany)

P22. Immunomodulation induced by resveratrol or genistein on proliferation and apoptosis of tumour colon cells
L I Brasoveanu (Romania)

P23. Efficient ex vivo lysis of acute myeloid leukaemic (AML) cells mediated by triplebodies with dual-targeting capability in conjunction with natural killer cells as effectors
G Fey (Germany)

P24. Aviscumine enhances NK-cytotoxicity against tumor cells
G Gamerith (Austria)

17:15-18:45 Poster session: Other

P25. Access to diagnostics: A bottleneck for immunotherapeutics development – case example of MAGE-A3 cancer immunotherapeutic
K Lykopoulos (UK)

P26. Cancer associated fibroblasts contribute to the immune suppression of breast cancer by augmentation of the inflammatory products
L Langroudi (Iran)

P27. Alemtuzumab (anti-CD52 monoclonal antibody) as single-agent therapy in patients with relapsed/refractory chronic lymphocytic leukaemia (CLL) – a single region experience on consecutive patients
S Eketorp Sylvan (Sweden)

P28. Efficient CD19-positive leukaemia cell lysis mediated by a T cell-recruiting triplebody a[19-3-19]
C Roskopf (Germany)

P29. T-cell responses to oncogenic Merkel cell polyomavirus proteins distinguish Merkel cell carcinoma patients from healthy donors
S R Hadrup (Denmark)

P30. The use of HER2 receptors status as a prognostic index for estrogen receptor positive breast cancer patients
A Tawfeek (Iraq)

P31. Genomic profiling and functional characterisation of a new myeloid cell type enriched in renal cell carcinoma
D Brech (Germany)

P32. High resolution mass spectrometry reveals the depth and diversity of HLA-I peptidomes
M Bassani-Sternberg (Germany)

P33. NK-92 cellular immunotherapy as an alternative to donor derived peripheral blood NK cells
H Klingemann (USA)

THURSDAY 13 MARCH 2014

Plenary session: Targeting immune suppression – Chairs: J P Allison (USA) and S Khleif (USA)

08:30	S34. KEYNOTE LECTURE: Regulatory T cells and suppression of immune response* J P Allison (USA)
09:00	S2. Therapeutic strategies based on overcoming immune resistance mechanisms within the melanoma tumour microenvironment T Gajewski (USA)
09:20	S35. Investigating the ICOS/ICOSL pathway as a target for combination therapy with anti-CTLA-4 P Sharma (USA)
09:40	S37. Inhibition of indoleamine-2,3-dioxygenase (IDO)-mediated tumour immune escape* C Opitz (Germany)
10:00-10:30	Coffee break

Plenary session: Stimulation of immune function – Chairs: L Zitvogel (France) and A Dalgleish (UK)

10:30	S38. Many reasons for combining immunotherapy with chemotherapy to enhance anti tumour response A Dalgleish (UK)
10:50	S39. Role of gut microbiota in the anticancer effects of cytotoxic drugs* L Zitvogel (France)
11.10	Rationales, current and future for combination of immunooncology therapies* Speaker to be announced
11.30	S40. Oncolytic herpes simplex virus encoding GM-CSF for melanoma therapy* M Maiwald (Switzerland)
11.50	S41. Novel CEA-targeted IL2 variant immunocytokine for immunotherapy of cancer C Klein (Switzerland)
12:10-14:00	Lunch
13:00-14:00	Roche Satellite Symposium

Plenary session: Therapeutic anti-cancer vaccines – Chairs: J P Allison (USA) and S Endres (Germany)

14:00 S42. KEYNOTE LECTURE: Vaccine development*
S Khleif (USA)

14:30 S43. Dendritic cell vaccination combined with CTLA4 blockade
K Thielemans (Belgium)

14:50 S44. Role of CD40 activation in anti-tumour vaccine efficacy*
C J M Melief (the Netherlands)

15:10 S45. mRNA-based cancer vaccines*
K Kallen (Germany)

15:30-16:00 Coffee break

Plenary session: Monitoring of immunotherapy – Chairs: L Hakansson (Sweden) and B Fox (USA)

16:00 S46. Immune response parameters*
L Hakansson (Sweden)

16:20 S47. Tumour infiltrating lymphoctes - a predictive marker?
J Galon (France)

16:40 S48. Biomarker development for ipilimumab and prostate GVAX treatment
T De Gruijl (the Netherlands)

17:00 S49. Clinical activity and development of biomarkers for an engineered anti PDL1 antibody MPDL3280A
E Cha (USA)

17:20 S50. Proffered paper: Complex tumour microenvironment screening platform captures biological responses of cancer therapeutics
H Layman (USA)

17:30-19:00 Poster session: Vaccines

P51. Identification of prostate cancer-associated antigens by oxygen manipulation
T Ma (USA)

P52. A new pathway of tumour antigen loading of human dendritic cells via intercellular communication
G Penna (Italy)

P53. Effect of lymphodepletion and tumour on host and reconstituted regulatory T-cells in a model of murine melanoma
J Kovács (Germany)

P54. Possible role of MDSC for the induction of tumour-specific T cells following LRAST
P Rose (Germany)

P55. Dendritic cell vaccination for postremission therapy in AML
F Lichtenegger (Germany)

P56. A novel cancer vaccine with nanogel-based antigen transporter and sequence-optimised long peptide antigen
N Harada (Japan)

P57. Activation of RIG-I induces immunogenic cell death
S Bek (Germany)

17:30-19:00 Poster session: Immunocytokines

P58. Can T-cells predict response to intravesical BCG immunotherapy in high-risk non-invasive bladder cancer
S Jallad (UK)

17:30-19:00 Poster session: Combination therapy

P59. Depleting the suppressors for the benefit of immunotherapy against cervical cancer
O Draghiciu (the Netherlands)

P60. Microtubule-depolymerising agents used in antibody-drug-conjugates induce anti-tumour immunity by stimulation of dendritic cells
K Martin (Switzerland)

P62. Effectiveness of melatonin, IL-25 and siRNA IL-17B in growth control of breast cancer cell lines
G Bottaro Gelaleti (Brazil)

17:30-19:00 Poster session: Adoptive immunotherapy

P63. Autologous tumour cells and SW742 allogeneic cell line have comparable stimulating effect on PBMCs of gastrointestinal malignant patients in vitro
A Sheikhi (Iran)

P64. T cell re-direction against Glypican-3 for immunotherapy of hepatocellular carcinoma
C Dargel (Germany)

P65. Minor-histocompatibility-antigen UTY as target for graft-versus-leukaemia and graft-versus-haematopoiesis in the canine-model
D Bund (Germany)

P66. Generating and characterising WT1-specific T cells – research towards adoptive tumour therapy
S Schmied (Germany)

P67. Targeted natural killer (NK) cell based adoptive immunotherapy for the treatment of patients with non-small cell lung cancer (NSCLC) after radiochemotherapy (RCT) – clinical application of NK cells activated by heat shock protein 70 (Hsp70)
H Specht (Germany)

17:30-19:00 Poster session: Engineered T-cell therapy

P68. A new EGFR – EpCAM bispecific antibody enhances the efficacy of adoptive T-cell therapy in a murine gastric tumour model
S Kobold (Germany)

P69. Targeting naturally presented, leukemia-derived HLA ligands with TCR-transgenic T cells for the treatment of therapy refractory leukemias
R Klar (Germany)

P70. Development of clinically implementable imaging strategies for tracking T cell receptor-transgenic T cells
S Mall (Germany)

P71. Adoptive transfer of TCR gene-transduced lymphocytes targeting MAGE-A4 for refractory esophageal cancer
Y Miyahara (Japan)

P72. Transgenic expression of a chimeric signaling receptor to facilitate T cell costimulation in the tumour environment
R Schlenker (Germany)

P73. Functional characterisation of HBV-specific T cell receptors for redirection of T cells against HBV infected hepatocytes
K Krebs (Germany)

P74. A Good Manufacturing Practice procedure to generate therapeutic numbers of highly pure anti-leukaemic virus-specific T-cells
M Van Loenen (the Netherlands)

P75. Genetic engineering of T cells for increased homing to the tumor site
M Idorn (Denmark)

FRIDAY 14 MARCH 2014

Plenary session: Adoptive cell therapy – Chairs: F Marincola (Qatar) and to be announced

08:30	S75. KEYNOTE LECTURE: Basic concept for the understanding of cancer mediated immune rejection F Marincola (Qatar)
09:00	S76. Adoptive cell therapy of melanoma with autologous tumour infiltrating lymphocytes I M Svane (Denmark)
09:20	S77. Proffered paper: In vitro induced response patterns of antileukemic T-cells – characterization by combination of functional assays, spectratyping and next generation sequencing S Reuther (Germany)
09:40	S78. Proffered paper: High-affinity CD20-specific TCRs suitable for adoptive immunotherapy can be readily isolated from the allo-repertoire using reverse immunology L Jahn (the Netherlands)
10:00-10:30	Coffee break

Plenary session: Engineered T cell therapy – Chairs: A Shen (USA) and R Hawkins (UK)

10:30	S79. CAR strategies in solid tumours C Rossig (Germany)
11:00	S80. Prolongation of T cell response by OX40 co-signalling CARS H Abken (Germany)
11:20	S81. Proffered paper: A new PD1-CD28 chimeric receptor overcomes PD-1-mediated immunosuppression in adoptive T cell therapy S Kobold (Germany)
11:40	S82. Proffered paper: In-vivo testing of PSMA-targeted T-cell immunotherapy for prostate cancer Z Liu (UK)
12:00	Closing Remarks V Nuessler (Germany)

Journal for ImmunoTherapy of Cancer 2014, Volume 2 Suppl 2
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Journal for ImmunoTherapy of Cancer

MEETING ABSTRACTS Open Access

Abstracts of the 1st Immunotherapy of Cancer Conference (ITOC1)

Munich, Germany. 12-14 March 2014

Edited by Heinz Zwierzina and Volkmar Nuesslar

Published: 12 March 2014

These abstracts are available online at http://www.immunotherapyofcancer.org/supplements/2/S2

INVITED SPEAKER PRESENTATIONS

I1

S2. Therapeutic strategies based on overcoming immune resistance mechanisms within the melanoma tumour microenvironment

T Gajewski
University of Chicago, Department of Pathology, Chicago, IL, USA
Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I1

Two major categories of melanoma metastases have been observed based on gene expression profiling and confirmatory assays. One subgroup of patients has an inflamed phenotype that includes expression of chemokines, T cell markers, and other immunoregulatory factors. In contrast, the other major subset lacks this phenotype and appears to display immune "exclusion". The optimal immunotherapeutic intervention to gain clinical benefit may be distinct in these two global subsets. The T cell-inflamed tumor microenvironment subset contains the highest expression of negative regulatory factors, including PD-L1, IDO, and FoxP3$^+$ Tregs, and clear evidence for T cell-intrinsic anergy has also emerged. In addition, the mechanism of induction of these inhibitory mechanisms has been elucidated—PD-L1 and IDO are induced by IFN-γ, and Tregs are largely recruited by the chemokine CCL22, both being produced by activated CD8$^+$ effector T cells. Preclinical experiments have confirmed a critical role for all 4 of these mechanisms in limiting anti-tumor T cell efficacy in vivo, giving candidate treatment strategies for translation back into the clinic. These include anti-PD-1/PD-L1 mAbs, IDO inhibitors, and approaches to deplete CD25$^+$ Tregs and/or reverse anergy. The presence of multiple inhibitory mechanisms in the same tumor microenvironment argues that combination therapies may be advantageous to overcome compensatory effects. Preclinical data indicated synergy between anti-CTLA-4 +/- anti-PD-L1 +/- IDO inhibition. The mechanism of synergy is striking, as it correlates with a marked improvement of IL-2 production and proliferation of tumor-infiltrating CD8$^+$ T cells. Clinical translation of these combination immunotherapies is promising and ongoing. In contrast to the T cell-inflamed melanomas, a new paradigm may be needed to promote de novo inflammation in cases of the non-T cell-infiltrated tumor microenvironment. Natural innate immune sensing of tumors appears to occur via the host STING pathway, type I IFN production, and cross-priming of T cells via CD8α^+ DCs. New strategies are being developed to engage or mimic this pathway as a therapeutic endeavor.

I2

S5. Proffered paper: Maintenance therapy of metastatic colorectal carcinoma with the TLR-9 agonist MGN1703: clinical and immunological predictive pretreatment factors of activity in the IMPACT trial

H Schmoll[1*], BW Wittig[2], DA Arnold[3], JRK Riera-Knorrenschild[4], DN Nietsche[5], HK Kroening[6], FM Mayer[7], JA Andel[8], RZ Ziebermayr[9], WS Scheithauer[10]
[1]Universitätsklinikum HalleOnkologie/Hä, Halle, Germany; [2]Freie Universität Berlin, Foundation Institute Molecular Bioogy and Bioinformatics, Berlin, Germany; [3]Tumor Biology Center, Medical Oncology, Freiburg, Germany; [4]University Clinic Giessen/Marburg, Haematology and Oncology, Marburg, Germany; [5]Hospital Barmherzige Schwestern, Internal Medicine, Linz, Austria; [6]Schwerpunktpraxis, Hematology and Oncology, Magdeburg, Germany; [7]University Tübingen Medical Center, Internal Medicine II, Tübingen, Germany; [8]Landeskrankenhaus Steyr, Internal Medicine II, Steyr, Austria; [9]Elisabethinen, Hematology with stem cell transplantation and medical oncology, Linz, Austria; [10]Medical University Vienna, Internal medicine and comprehensive cancer center, Vienna, Austria
Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I2

Background: MGN1703 is a synthetic DNA-based immunomodulator acting as TLR-9 agonist which has shown preclinical activity in metastatic colorectal carcinoma (mCRC) as well as a good safety profile in patients with metastatic solid tumours in a Phase 1 trial. The IMPACT trial was conducted to assess clinical efficacy, safety, and immunological effects of MGN1703 as maintenance therapy twice weekly s.c. vs. placebo.
Methods: IMPACT was an international randomised (2:1) double-blind placebo-controlled phase 2 trial in patients with mCRC who achieved disease control (CR, PR, SD) after 4.5 to 6 months of 1st-line induction chemotherapy with FOLFOX/XELOX or FOLFIRI +/- bevacizumab. Due to slow recruitment the trial was prematurely closed in May 2012 after randomisation of 59 out of 129 planned patients (43 received MGN1703, 16 placebo).
Results: There was evidence of a superior effect of MGN1703 compared with placebo. The hazard ratio (HR) for the primary endpoint PFS on maintenance treatment group was 0.55, (p=0.040) on local assessment and 0.56 (p=0.070) by independent radiological review. Notably, at time of study closure 4 patients receiving MGN1703 were still free of progression and continued treatment in compassionate use protocols.
Exploratory uni- and multivariate Cox regression analyses showed a possibly predictive effect of baseline CEA level and tumour size change

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during first-line induction therapy. HR was 0.07 (p<0.0001) for patients with normal CEA level and 0.39 (p=0.005) for patients with an objective response to induction chemotherapy.
A predefined analysis was performed on immunological cell populations at baseline and during the study. This allowed to confirm activation of innate immune system effector cells in patients receiving MGN1703. Cox regression and receiver operating characteristic (ROC) analyses identified the presence at baseline of activated NKT-cells (CD3+, CD56+, CD69+) as potentially predictive of benefit from MGN1703 treatment. The HR was 0.27 (p=0.007) using a cut-off value for activated NKT-cells of 3.08%.
Conclusions: After induction chemotherapy for mCRC, maintenance with MGN1703 is associated with improved PFS compared to placebo and low toxicity.
We found preliminary evidence that pretreatment CEA plasma levels, tumour response and activated NKT cells counts may allow identifying patients benefiting most from MGN1703 maintenance therapy. A confirmatory clinical study in patients with mCRC is planned to start in 2014.

I3

S6. Proffered paper: Anti-tumour immunomodulatory activity of heat shock protein Hsp70 in therapy of malignant brain tumours: preclinical and clinical data

M Shevtsov[1*], A Kim[2], W Khachatryan[2], A Pozdnyakov[3], I Romanova[4], I Guzhova[1], B Margulis[1]
[1]Institute of Cytology RAS, Saint-Petersburg, Russian Federation; [2]A.L. Polenov Russian Scientific Research Institute of Neurosurgery, Paediatric Neurosurgery, Saint-Petersburg, Russian Federation; [3]City Clinical Oncological Dispenser, Department of Radiology, Saint-Petersburg, Russian Federation; [4]I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, Department of Morphology, Saint-Petersburg, Russian Federation
Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I3

Background: Immunotherapy provides a specific anti-tumour activity with minimal side effects and thus could be used in treatment of malignant brain tumours. Molecular chaperone Hsp70 is well known for its ability to stimulate innate and adaptive anti-cancer immune response. In in vivo studies we proved the efficacy of the intratumoral delivery of Hsp70 in the model of intracranial glioma C6 in animals. Based on our preclinical *in vivo* data we conducted for the first time pilot study for assessment of anti-tumour activity of Hsp70 in patients with brain tumours.
Material and methods: 12 patients with diagnosis of malignant brain tumour were enrolled in clinical trial. Following tumour resection protein was infused into tumor cavity. Specific immune response was evaluated in delayed hypersensitivity test (DTH). Peripheral blood was monitored for possible changes in lymphocyte subpopulations cytokine levels and cytolityc activity of NK-cells. Additionally cytokine production was analyzed in dynamics in liquor after injection of Hsp70. All patients were monitored for adverse effects.
Results: Intratumoral injections of Hsp70 were well tolerated in patients. One patient had complete clinical response and one partial response documented by radiological findings. In three patients we observed positive DTH-test. In peripheral blood we observed a shift from cytokines of those provided by Th_2-helpers towards cytokines from Th_1-mediated response (i.e., INF-gamma, TNF-alpha). This data corresponded to changes in lymphocyte subpopulations. NK-cell activity was not altered.
Conclusions: This pilot study demonstrated for the first time feasibility and safety of intratumoral delivery of recombinant Hsp70 in cancer patients. Based on our preclinical and clinical findings we proposed the novel hypothesis of immunomodulatory mechanism of Hsp70. Our results suggest that purified Hsp70 can induce specific effective anti-tumor immune response and warrants further investigation in randomised clinical trials.

I4

S7. KEYNOTE LECTURE: Immunobiological properties of the human cancer stem cells

C Maccalli[*], EZ Elisabetta Zambon, FC Filippo Capocefalo, GP Giorgio Parmiani
San Raffaele Scientific and University Institute, Unit of Immunbiotherapy of Melanoma and Solid Tumors, Department of Molecular Oncology, Milan, Italy
Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I4

Background: Cells with "stem cell properties", denominated cancer stem cells (CSCs), have been recently isolated from a variety of human solid tumors. These cells have been considered as responsible of resistance to standard therapy such as chemotherapy and radiotherapy. Immunotherapy, due to its specificity and lack of toxicity can represent a promising approach to target cancer stem cells.
Thus, it is relevant to assess whether CSCs isolated from solid tumors, can be exploited as source of antigens to elicit T cell-mediated immune responses and to design novel immunotherapeutic protocols for tumors.
Results: In our group CSCs from glioblastoma multiforme (GBM) and colorectal cancer (CRC) have been isolated from tumor biopsies and have been biologically characterized. Moreover, a detailed immunological characterization of these CSCs has been performed leading to the identification of a low immunogenic profile with negative immunoregulatory properties (*Di Tomaso et al, 2010*).
We found that both CSCs and the differentiated counterpart of tumors (FBS tumor cells) expressed immune modulatory molecules, such as CTLA-4, PD-1, PDL-1 and B7H3, with, in some cases, higher levels in CSCS vs. FBS tumor cells. Furthermore, a differential gene signature that was confirmed at the protein level for some immunological-related molecules was also found for CSC and FBS lines. A candidate negative immunoregulatory molecule is represented by the indoleamine 2,3-dioxygenase (IDO), a molecule implicated in the generation of immune tolerance. By RT-PCR we detected the preferential increase of the mRNA of this molecule in CSCs vs FBS tumor cells following IFN-⍰ treatment. The functional activity of IDO determining, by a colorimetric assay, IDO-mediated tryptophan catabolism in culture supernatants was also found to be preferentially associated with CSCs.
Of note, CRC CICs expressed higher levels of IL-4 as compared with FBS tumor cell pairs. CIC-associated IL-4 could mediate, by cell-to cell contact, the inhibition of proliferation of T cells following the co-culture with autologous CIC.
The neutralization in functional assays of at least one of the negative modulatory signals mentioned above, led to the modulation of both the type and the quality of tumor-specific T cell immune responses.
Micro-RNA (miRNA) profile, including miRNA with immunoregulatory functions, has been identified to be specifically associated with CSCs vs. FBS tumor cell pairs as well.
Conclusions: Multiple mechanisms of immunoregulation that are exploited by CSCs to escape from T cell-mediated surveillance have been identified. These results may allow designing more effective immunotherapy protocols to target CSCs from GBM and/or CRC patients.

I5

S9. Proffered paper: Identification of novel immune checkpoints as targets for cancer immunotherapy

G Cojocaru[*], G Rotman, O Levy, A Toporik, L Dassa, I Vaknin, S Sameah-Greenwald, I Barbiro, E Neria, Z Levine
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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I5

Members of the B7/CD28 family of immune checkpoints, such as CTLA4, PD1 and PDL-1, play critical roles in T cell regulation and have emerged as promising drug targets for cancer immunotherapy. We hypothesize that additional novel members of the B7/CD28 family play a role as negative immune regulators and thus may serve as targets for therapeutic mAbs. Utilising Compugen's predictive discovery platform, we identified nine novel members of this family that may serve as immune checkpoints. The therapeutic relevance of three of these proteins, CGEN-15001T, CGEN-15022, and CGEN-15049, was confirmed following the validation of their immunomodulatory properties and their expression in various cancers. Two of these proteins, CGEN-15001T and CGEN-15022, are the basis of a license and collaboration agreement recently signed with Bayer as targets for cancer immunotherapy. Here we present results obtained for an additional novel immune checkpoint, CGEN-15049. Following its ectopic expression on cancer cell lines, CGEN-15049 inhibits the activity of NK cells and cytotoxic T cells (CTLs). The fusion protein, consisting of the extracellular domain of CGEN-15049 fused to an IgG Fc domain, displays robust inhibition of T cell activation and enhances iTregs differentiation. IHC studies indicate that CGEN-15049 is expressed in tumour cells of numerous types of cancers, as well as in tumour infiltrating immune cells. Based on its immunomodulatory

activities on several types of immune cells which play key roles in cancer immune evasion, together with its expression pattern,CGEN-15049 may serve as mAb target for cancer immunotherapy.

I6

S35. Investigating the ICOS/ICOSL pathway as a target for combination therapy with anti-CTLA-4

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I6

Biomarker studies used with immunotherapeutic strategies in the clinic have typically involved monitoring immunologic changes within the systemic circulation; however, recent data indicate that immunological changes within tumor tissues will be more likely to predict clinical responses. To obtain such data we conducted the first pre-surgical clinical trial with anti-CTLA-4 (ipilimumab) in a cohort of patients with localized bladder cancer. We are also conducting the first combination therapy pre-surgical trial with ipilimumab plus leuprolide acetate in patients with localized prostate cancer. Immunological data from these trials are obtained from both tumor tissues and blood samples.

We found an increased frequency of CD4 and CD8 T cells expressing high levels of inducible costimulator (ICOS) and decreased frequency of FOXP3-expressing CD4 T cells within tumor tissues of treated patients. The $CD4^{+}ICOS^{hi}$ population contained effector T cells that produced IFN-g and recognized the cancer-testis antigen NY-ESO-1 expressed on tumor cells. We therefore identified ICOS as marker of a subset of effector T cells that is increased in frequency after anti-CTLA-4 therapy. $ICOS^{+}$ T cells are being explored as both a pharmacodynamic marker for treatment with anti-CTLA-4 as well as a novel target to improve the efficacy of anti-CTLA-4 therapy.

These observations led us to test the possibility that engagement of ICOS could enhance the efficacy of anti-CTLA-4 therapy. To this end we transduced mouse B16F10 melanoma cells with a cDNA encoding ICOSL or a control construct. B16-ICOSL+ cells (IVAX) and control B16 cells were irradiated and used alone or in combination with anti-CTLA-4 to treat mice bearing established B16F10 tumors. We found that combination of the IVAX with anti-CTLA-4 was markedly more effective than the control vaccine plus anti-CTLA-4 or that of any single treatment alone. The increase in therapeutic efficacy was accompanied by a marked in increase in the density and functionality of CD4 and CD8 T cells within the tumor.

These results suggest a novel strategy for manipulating the immune system to enhance anti-tumor responses: checkpoint blockade coupled with provision of agonist signals to enhance costimulation mediated by ICOS.

I7

S38. Many reasons for combining immunotherapy with chemotherapy to enhance anti tumour response

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I7

Background: Chemotherapy and Immunotherapy were always considered incompatible in the treatment of cancer. However, analysis of sequential use has revealed that there may be significant additive potential if not synergistic, in enhancing the immune response to cancer.

Material and methods: There are many reasons why chemotherapy may enhance immune therapy. These include1) the release of tumour antigens to a primed immune response, 2) the suppression and reduction of suppressor and regulatory T cells in the tumour and stromal tissues, 3) The priming of tumour cells to make them more visible to the immune response, 4) reduction of inflammatory and growth factor drive.

Results: Enhancing the immune response prior to chemotherapy and releasing antigens as well as stimulating the immune response after Chemotherapy or radiotherapy are both effective models. Here we look at specific examples such as pre-stimulation with non specific mycobacterial vaccines that will enhance the responses to gemcitabine and zometa, the former boosting the Antigen specific responses and the later the gamma delta T cell activity.

Low dose chemotherapy such as with cyclophosphamide or pre vaccine treatment with the IMiDs such as Revlimid have been shown to enhance the immune response directly (co-stimlation) and indirectly by depressing T reg function. Both agents are in therapeutic vaccine trials for both HIV and Cancer. Another agent which is anti-inflammatory and immune stimulatory is low dose naltrexone (LDN) and has been noted to induce vitiligo in melanoma patient. This has lead to studies showing that in addition to modulating opiate receptors on immune cells it is a strong interactor with TLRs.

Conclusions: There are numerous reasons how chemotherapy and immunotherapy can be additive if not synergistic. The sequential use of these agents may be more or as important as combining them, and the availability of the check point blockers has added another dimensions as well as the resurrection of cytokines such as low dose IL-2 for keeping the effector/memory cells active. There is a good case to prime with non specific vaccine activators, to then treat with chemotherapy followed by check point inhibitors and them low dose cytokines , such as IL-2 or other candidates such as IL-7,12,15, or 21.

I8

S41. Novel CEA-targeted IL2 variant immunocytokine for immunotherapy of cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I8

Background: Here we describe a novel class of monomeric tumor-targeted immunocytokines that comprise a single IL-2 variant (IL2v) with abolished CD25 binding that is fused to the C-terminus of a tumor specific antibody with a heterodimeric Fc devoid of FcγR and C1q binding. For tumor targeting, human/humanized high affinity antibodies against CEA (GA504) or FAP (GA501) were selected.

Materials & methods: CEA- and FAP-IL2v activity was tested on effector cells by assessing the activation of P-STAT5, cell proliferation, sensitivity to Fas-induced apoptosis, expression of activation markers and cytokine release upon treatment. Safety, pharmacokinetics, pharmacodynamics and anti-tumor efficacy were analyzed in fully immunocompetent (CEA transgenic) C57Bl/6 mice as single agent and in combination with ADCC competent antibodies in SCID/hCD16 tg mice as well as Balb/neuT genetically engineered mice. Tumor targeting was investigated in the orthotopic syngeneic Renca RCC model in Balb/c mice.

Results: FAP- and CEA-IL2v completely lack binding to CD25, but retain IL-Rβγ binding, and show pM binding affinity to respective antigens, FAP or CEA. As a consequence of abolished binding to CD25 they do not preferentially activate Tregs, but IL-2Rβγ mediated activity is retained and FAP- and CEA-IL2v activate NK, $CD4^{+}$ and $CD8^{+}$ T cells as shown by induction of activation markers, cell proliferation and cytokine release. Furthermore, CEA-IL2v and FAP-IL2v enhance the cytotoxic activity of NK cells when combined with ADCC-competent antibodies. Mechanism of action studies in fully immunocompetent mice showed that the molecules strongly expand and activate NK, $CD8^{+}$ T cells and gd T cells (up to 100-fold) and skew the CD4:CD8 ratio strongly towards $CD8^{+}$ T cells in the peripheral blood, lymphoid tissues, and in the tumor. In C57Bl/6 mice. MicroSPECT/CT imaging with radioactively labeled FAP-IL2v reveal good FAP-mediated tumor targeting in the orthotopic syngeneic Renca model with low normal tissue uptake and low accumulation in lymphoid tissues, contrary to analogous IL-2 based immunocytokine that shows preferential targeting to lymphoid tissue. Studies in tumor-bearing mice show dose-dependent anti-tumor efficacy of CEA-IL2v in syngeneic MC38-CEA and PancO2-CEA models. Combination of a tumor-stroma targeted TNCA-IL2v with an ADCC-competent ratHER2 antibody in the Balb/neuT spontaneous breast cancer model results in enhanced antitumoral efficacy.

Conclusion: Compared to classical IL-2-based immunocytokines, CEA- and FAP-IL2v demonstrate superior safety, PK and tumor targeting due to abolished CD25 binding, monovalency and high-affinity to tumor antigens while failing to preferentially induce Tregs. CEA- and FAP-IL2v retain the capacity to activate and expand NK and CD8+ effector T cells both in the periphery and tumor microenvironment supporting their further nonclinical

and clinical investigation for immunotherapy of cancer. Clinical trials with CEA-IL2v are foreseen in 2014.

I9

S43. Dendritic cell vaccination combined with CTLA4 blockade

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I9

Background: Electroporation of DC with mRNA also allows the loading of these cells with tumor antigens and the functional modification of the cellular vaccine. To this goal, we provide three different molecular adjuvants to immature, monocyte derived DCs through electroporation with mRNA coding for CD40L, CD70 and caTLR4 or so-called TriMix mRNA.
At our institution, clinical trials in pretreated advanced melanoma patients are being performed. These patients are treated with **TriMixDC-MEL**, a mixture of TriMix-DC co-electroporated with mRNA encoding a fusion of DC.LAMP and 1 of 4 melanoma associated antigens (gp100, tyrosinase, MAGE-C2 or MAGE-A3).
Results: In a pilot clinical trial, 24.10^6 TriMixDC-MEL cells were administrated solely by the intradermal (ID) route. Subsequently, a phase IB was conducted to investigate the safety of administrating TriMixDC-MEL by the intravenous (IV) and ID-route. ID administration of TriMixDC-MEL was found to be feasible, safe, effectively stimulating $CD8^+$ T-cell responses, but did not result in objective tumor responses. In contrast, the combined ID/IV administration is associated with distinct but manageable side-effects and has seemingly superior clinical activity as compared to DC administered solely ID in patients with pretreated advanced melanoma. We also investigated the safety and activity of TriMixDC-MEL combined with ipilimumab. The best objective tumor response observed in this trial (37 evaluable pts) were 6 CR, 6 PR, 7 SD and 16 PD (disease control rate: 51%). All 6 CR and 3 PR are currently ongoing (respectively after 23, 22, 20, 20, 19, 17, 15, 14, 14 months). This phase II study of TriMixDC-MEL ID/IV in combination with ipi demonstrates anti-melanoma activity in over 50% of the patients with therapy resistant advanced melanoma.
Conclusion: Further clinical development of TriMixDC-MEL in combination with immune checkpoint modulators is warranted.
Furthermore, TriMixDC-MEL is currently under evaluation in a randomized phase II trial in the adjuvant setting following resection of macrometastases.

I10

S47. Tumour infiltrating lymphocytes - a predictive marker?

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I10

Introduction: To date the anatomic extent of tumor (TNM classifications) has been by far the most important factors to predict the prognosis of cancer patients. However, this classification provides limited prognostic information in estimating the outcome in cancer and does not predict response to therapy.
Materials and methods: large-scale approaches, and quantitative measurements, we evaluated the importance of the host-immune response within human tumors.
Results: We showed that tumors from human colorectal cancer with a high density of infiltrating memory and effector memory T-cells (TEM) are less likely to disseminate to lymphovascular and perineural structures and to regional lymph-nodes. We showed that the combination of immune parameters associating the nature, the density, the functional orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host immune reaction on patients prognosis. We defined these parameters as the "immune contexture", and factors modulating it will be discussed. Based on the *immune contexture*, a standardized, simple and powerful immune stratification system, termed the *"Immunoscore"*, was delineated that may bear a prognostic power superior to that of the currently used cancer staging system. Tumor invasion parameters were statistically dependent on the host-immune reaction. A worldwide Immunoscore consortium is testing the prognostic value of the Immunoscore, using a standardized assay to routinely measure the immune status of a cancer patient.
Conclusions: The functional orientation of the *immune contexture* is characterized by immune signatures qualitatively similar to those predicting response to immunotherapy, Thus, the continuum of immune response existing, spanning a balance between tumor cell growth and elimination, will be discussed.

I11

S48. Biomarker development for ipilimumab and prostate GVAX treatment

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I11

Background: Immunotherapeutic approaches such as vaccination or immune checkpoint blockade have proven to be clinically active in prostate cancer, but only in fractions of treated patients; this calls for personalized application of these novel therapies based on predictive biomarkers.
Methods: Our own research over the past years has focused on the clinical efficacy in patients with castration-resistant prostate cancer of the combination of an allogeneic cell line-based vaccine (Prostate GVAX) and an anti-CTLA4 checkpoint inhibitor (ipilimumab) in a Phase-I/II dose escalation/expansion trial. We carried out an extensive immune monitoring programme comprising flowcytometric profiling of lymphoid and myeloid subsets in peripheral blood (PB) and T cell and serological reactivity to a panel of known tumor antigens, all before and after treatment.
Results: On-treatment PSA declines of more than 50% were observed in 5, and PSA stabilizations in 12 of 28 patients. Regressing bone and lymph node metastases were observed in 2/5 responding patients. Significantly prolonged overall survival (OS) was observed for patients with high pre-treatment frequencies of CD4+CTLA-4+, CD4+PD-1+, or differentiated CD8+ T cells, or low pre-treatment frequencies of regulatory T cells. Treatment-induced activation of PB Dendritic Cell subsets was similarly associated with significantly prolonged OS. In contrast, high pre-treatment frequencies of monocytic Myeloid-Derived Suppressor Cells (MDSC) were associated with reduced OS. Th2/Th17 cytokine profiles were induced. Indeed, profound up-regulation of CD4+IL-5+ T cell frequencies was associated with improved OS ($p=0.03$) and correlated significantly with the breadth of the induced antibody response. IgG antibody responses against 11 (prostate) tumor-associated antigens were determined and increased seroreactivity to prostate-specific membrane antigen (PSMA), pyridoxamine 5'-phosphate oxidase (PNPO) and/or Neuropilin-2 (NRP2) was significantly correlated with improved OS ($p=0.002$ for combined upregulated seroreactivity to all three). Finally, patients with pre-existing NY-ESO-1 T cell reactivity also demonstrated a significantly prolonged OS ($p=0.044$).
Conclusion: Together these data provide an immune profile to predict clinical outcome. Importantly, cluster analysis revealed pre-treatment expression of CTLA-4 by circulating CD4+ T cells and an immune-stimulatory myeloid profile to be dominant predictors for OS after Prostate GVAX/ipilimumab therapy. These flowcytometry-based parameters may thus provide potentially useful and easy-to-use biomarkers for patient selection.

I12

S50. Proffered paper: Complex tumour microenvironment screening platform captures biological responses of cancer therapeutics

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I12

The tumour microenvironment consists of a heterogeneous population of cancer-associated fibroblasts, leaky vasculature, polarised and suppressed immune cells, and hyperproliferative, transformed and invasive epithelial cells. Capturing the complex phenotypic biology of the tumour microenvironment *in vitro* is extremely challenging as numerous components drive a malignant phenotype. Currently, cancer drug screening is limited by lengthy clinical

trials, rodent models unable to capture human biology, and *in vitro* screening in monoculture which ignores complex cell interactions. An *in vitro* co-culture platform to mirror these cancer microenvironments may permit phenotypic screening of multiple cancer drugs to elucidate additive or synergistic effects prior to clinical trials.

We have recently developed a panel of primary human co-cultures, BioMAP® Oncology Systems, which mimic the complexity of host-tumour stromal and vasculature microenvironment. By using primary human fibroblast or endothelial cells with stimulated peripheral blood mononuclear cells (PBMCs) and the human epithelial adenocarcinoma cell line HT-29, we are able to successfully model a stromal or vascular tumour microenvironment. These co-culture models allow us to generate a unique profile of biological responses for drugs tested at multiple concentrations. These biological responses are a function of 41 cell-based and soluble protein readouts, measured by immune-based methods, examining tumour-associated immunomodulation, angiogenesis, matrix remodeling, and cellular proliferation. Using paclitaxel as a model cancer drug we found that it exhibited immunostimulatory characteristics through upregulation of soluble GranzymeB, TNFα, and IFNγ. Upregulation of these readouts is found in the clinic consistent with cancer cell death via T-cell-dependent anti-tumour effects. We also screened typical standards of care (SOC) for colon cancer, gemcitabine and carboplatin, to determine their response profiles. Alternatively, these systems can be used to screen potential cancer drugs in combination or matrix format to assess therapeutic strategies for synergistic or additive effects. Profiling of cancer immunotherapeutics (e.g ipilimumab) in combination with current SOC provides insightful data for clinicians to understand immune-preserving and cancer-cell killing activities.

Profiling of compounds across a panel of tumour microenvironment co-cultures provides a powerful experimental platform in primary human cell-based system that allows researchers in early compound development to investigate their drug's potential therapeutic targets, and, at late-stage development, accelerate a drug(s) track through clinical testing. The ever increasing number of clinical trials using multiple cancer drugs can be reduced by utilising a matrix of drugs administered in our newly developed BioMAP® oncology systems to determine which drugs in combination are of clinical efficacy.

I13

S75. KEYNOTE LECTURE: Basic concept for the understanding of cancer mediated immune rejection

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I13

Background: Cancer is a multi-genic, complex biological phenomenon and its growth is affected by various categories of factors including the genetics of the host, the accumulating genetic alteration within cancer cells and environmental modifiers.

Material and methods: Therefore, to identify the determinants of immune-mediated tumor rejection during immunotherapy, a systematic and comprehensive monitoring approach needs to be applied that covers the various categories at a time relevant to the therapeutic intervention. In addition, a temporal dimension needs to be added to evaluate in real time the changes induced by treatment and their relationship with clinical outcome.

Results: In this presentation, we will review the salient concepts that should guide the future monitoring of clinical trials taking advantage of novel and comprehensive technological advances presenting examples of integrated approaches for the assessment of patients' response to adoptive T cell therapy.

Conclusions: Our experience highlights the need to apply multi-parametric approaches for the understanding of the mechanism(s) leading to cancer rejection by the immune system in humans.

I14

S76. Adoptive cell therapy of melanoma with autologous tumour infiltrating lymphocytes

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I14

Background: Adoptive T-cell therapy (ACT) with tumor infiltrating lymphocytes (TILs) is a personalized treatment for cancer defined as the infusion of T cells isolated from the patient's own tumor tissue after ex vivo activation and several rounds of expansion. This treatment has achieved impressive clinical results in several single institution phase I/II clinical trials performed outside Europe, and holds the promise to enter the mainstream of standard melanoma care in the near future. However, although transient, the toxicities associated with high-dose IL-2 classically administered together with TILs are severe and recent data have questioned its use.

Material and methods: In an ongoing phase I/II study, we have enrolled patients with progressive metastatic melanoma. TILs infusion was preceded by standard lymphodepleting chemotherapy but followed by low-dose subcutaneous IL-2 for 14 days or by an intravenous intermediate dose IL-2 decrescendo regimen.

Results: The lower doses of IL-2 considerably decreased the toxicity of the treatment while PET-CT imaging showed a preserved objective response rate of 48% including long-lasting complete responders. The absolute number of tumour specific T-cells infused was significantly associated to clinical response, with induction of peripheral tumour reactive T cells. To characterize the fate of TILs after infusion, we performed a longitudinal analysis of bulk tumor-reactive T cells from in vitro cultured TILs and found durable persistent T-cells after up to 1.5 years after infusion. Extensive analysis of disease relapse in two patients revealed multiple potential mechanisms of tumor recurrence, linked either to tumor-changes or immune response decline.

Conclusion: Despite its clinical efficacy, with impressive response rates and a significant fraction of long surviving complete responders, the implementation of TIL based ACT into current practice has been severely hampered by the technical complexity of cell production, the toxicity profile demanding treatment at specialize cancer centers, and lack of investment from the pharmaceutical industry. Next step should be a pivotal phase III trial in melanoma which is required for regulatory approval. Further improvement of the therapy could also be pursued through combination treatment.

I15

S77. Proffered paper: In vitro induced response patterns of antileukemic T-cells – characterization by combination of functional assays, spectratyping and next generation sequencing

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I15

Background: T-cell receptor (TCR) diversity is characterised by somatic alterations in the complementary determining region 3 (CDR3) of the human TCR β-chain. Complemented with the TCR alpha-chain, TCR diversity can hypothetically result in up to 10^{18} different TCR molecules.

Myeloid leukaemic cells can be induced to differentiate into leukaemia-derived dendritic cells (DC_{leu}) regaining the stimulatory capacity of professional DCs while presenting the whole leukaemic antigen repertoire.

Our aim was to identify TCR Vβ-chain-rearrangements in T-cells stimulated with leukaemic blasts and DC_{leu} in 3 patients with AML and furthermore to detect, amplify or monitor T-cell clones with defined Vβ-profiles in correlation with antileukaemic function, in vitro and in vivo.

Material and methods: HLA matched or HLA haplo-identical (allogeneic) donor- or autologous T-cells were repeatedly stimulated, either with leukaemic blasts or the corresponding DC_{leu} from 3 different AML-patients. Cytotoxicity assay was carried out for measuring the lytic activity of effector T-cells, spectratyping was performed to identify the restriction of the TCR Vβ-repertoire in unstimulated and stimulated T-cells and Sanger sequencing to analyse the β-chain sequence information including the CDR3 regions. Additionally next generation sequencing (NGS) was established to analyse the accurate TCR sequence information of thousands of TCR β-chains with high coverage.

Results: No significant differences in T-cell proliferation were observed. The T-cell mediated cytolytic response patterns showed blast lysis (n=1) and blast proliferation (n=2).

Spectratyping revealed a remarkable TCR Vβ-restriction of the $CD4^+$- or $CD8^+$-TCR repertoire of blast- or DC/DC_{leu}-stimulated T-cells, independently of blast or DC/DC_{leu} used as stimulators. Although in absolute terms, DC/DC_{leu} stimulation induced the highest grade of restriction in the $CD8^+$ T-cell subset, the $CD4^+$ T-cells seemed to be relatively more affected.
In vitro stimulation with DC/DC_{leu} resulted in an identical TCR (β-chain restriction pattern) as identified in vivo in a patient sample 3 months after allogeneic stem cell transplantation (SCT).
Conclusion: A combined strategy using spectratyping and NGS with functional tests may provide useful information about the specificity and efficacy of the intra-individual variable induced T-cell response.
Spectratyping allows the identification of a restricted Vβ-repertoire by Gaussian-like distribution, NGS allows sequencing of TCR repertoires with high coverage, novel software allows the analysis of the exact length and sequence composition (the combination of the Vβ- and Jβ-genes) of the β-chains, especially of the CDR3, and the exclusion of non-functional transcripts.
The identification of defined Vβ-T-cell clones may lead to selection procedures generating Graft-versus-Leukaemia reaction- but not Graft-versus-Host disease- mediating T-cells for adoptive immunotherapy after SCT.

I16

S78. Proffered paper: High-affinity CD20-specific TCRs suitable for adoptive immunotherapy can be readily isolated from the allo-repertoire using reverse immunology

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I16

Studies using T-cell receptor (TCR) or chimeric antigen receptor (CAR) transduced T-cells have shown the effectiveness of adoptive immunotherapy to treat different malignancies. The efficacy and safety of such interventions greatly depends on good target selection to prevent on-target toxicity. Furthermore, the broad application of TCR-based adoptive immunotherapy is hampered by a lack of an effective immune response against self-antigens. Through self-tolerance, T-cells carrying high-affinity TCRs reactive to self-antigens are deleted during thymic selection. An attractive strategy is to exploit the immunogenicity of foreign human leukocyte antigen (HLA) molecules to generate an effective immune response against these antigens. Here, we describe a protocol to efficiently isolate high-avidity alloHLA-restricted T-cells targeting the B-cell compartment.
From a B-cell peptide elution library 15 peptides derived from genes exhibiting B-cell restricted expression patterns were identified and peptide-MHC multimers (pMHC) of HLA-A*0201 were generated. Via MACSorting and FACSorting a plethora of pMHC-multimer binding T-cell clones from HLA-A*0201-negative individuals were isolated. Generated T-cell clones were selected based on peptide-specificity and avidity for further characterization. We successfully isolated two distinct T-cell clones carrying high-affinity TCRs specific for a CD20 peptide presented in HLA-A*0201. CD20 dependent recognition could be demonstrated by genetically engineering CD20-negative K562-A2 cells to express CD20. The isolated T-cell clones efficiently recognized CD20-expressing HLA-A*0201 primary chronic lymphocytic leukaemia (CLL), acute lymphoblastic leukaemia (ALL) and mantle cell lymphoma (MCL), while recognition of CD20-negative hematopoietic and non-hematopoietic cell-subsets was absent. In addition, the CD20-specific T-cell clones were able to more efficiently recognise ALL cell-lines than CD20 specific antibodies. We demonstrated that on ALL cell lines with only very low CD20 surface expression, the CD20-specific T cell clones could still efficiently recognise endogenously processed CD20-derived peptides in the context of HLA-A*0201.
In conclusion, we developed a platform for the rapid identification of high-affinity TCRs of therapeutic relevance targeted to self-antigens by combining gene expression data with valuable information on peptide processing from peptide elution studies and exploiting the immunogenicity of foreign HLA. Using this platform we successfully isolated CD20-specific TCRs which could broaden the application of immunotherapies targeted to CD20 in cases were CD20-cell surface expression is low. Based on its general principle the developed platform could easily be adapted to target other malignancies.

I17

S79. CAR strategies in solid tumours

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I17

The development of immune-based strategies for cancer for solid cancers is challenged by the scarcity of T cells with high receptor avidity for tumor-specific antigens within the patient´s lymphocyte repertoire, and by the failure of tumor cells to present antigen to T cells. Both obstacles can be bypassed by genetic modification of T cells with recombinant chimeric receptors (CARs) which redirect T cells towards a tumor surface antigen independent of antigen presentation. CAR reengineered T cells efficiently interact with tumor cells in vitro and have significant in vivo activity against tumor xenografts. Recently, first clinical trials have shown evidence for a potent antitumor activity of CD19-specific CAR T cells in leukemia. Current efforts focus on improving in vivo survival, functional persistence and potency of adoptively transferred anti-tumor T cells. The design of more effective strategies against both solid tumors and leukemias further depends on enhanced knowledge of specific mechanisms of immune escape. Moreover, rational combinations of targeted therapies with immunotherapies and optimal integration of cellular therapies into current treatment regimens may allow higher rates of durable responses.

I18

S80. Prolongation of T cell response by OX40 co-signalling CARS

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I18

Adoptive therapy of cancer with genetically redirected T cells showed spectacular efficacy in recent trials. A body of pre-clinical and clinical data indicate that young effector and central memory T cells perform superior in a primary anti-tumor response; repetitive antigen engagement, however, drives T cell maturation to terminally differentiated cells associated with the loss of CCR7 which enables T cells to persist in peripheral tissues. Chimeric antigen receptor (CAR) engineered $CCR7^-$ T cells more efficiently accumulated at the tumor site, secreted more IFN-g, expressed higher amounts of cytotoxic molecules and showed superior tumor cell lysis compared to the younger $CCR7^+$ cells. $CCR7^-$ T cells, however, were more prone to spontaneous and activation induced cell death which could be counteracted by simultaneous CD28 and OX40 (CD134) costimulation. Consequently, the combined CD28-z-OX40 signaling CAR rescued $CCR7^-$ T cells from apoptosis which then produced more efficient anti-tumor efficacy than $CCR7^+$ T cells redirected by the same CAR. In contrast, cytokine induced killer (CIK) cells, predominantly consisting of terminally differentiated $CD8^+CD56^+$ cells, accelerated terminal maturation of $CD56^+$ CIK cells producing high frequencies in activation induced cell death (AICD) and reduced anti-tumor efficiency when stimulated by the CD28-z-OX40 CAR compared to the CD28-z CAR. Translated into therapeutic strategies, T cell therapy will benefit from combined CD28-z-OX40 stimulation in the long-term by rescuing continuously generated $CCR7^-$ T cells for an anti-tumor attack. CAR redirected CIK cells benefit from CD28 co-stimulation; "super-costimulation" by the CD28-z-OX40 CAR, however, performed less in anti-tumor efficacy due to increased AICD.

I19

S81. Proffered paper: A new PD1-CD28 chimeric receptor overcomes PD-1-mediated immunosuppression in adoptive T cell therapy

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I19

Background: Although tumour-specific cytotoxic T cells are capable of killing tumour cells both *in vitro* and *in vivo*, treatment with adoptive

T cell transfer does not lead to sufficient tumour regression without adjuvant therapy. Tumour-promoted T cell exhaustion and anergy have been proposed to contribute to this lack of efficacy. We and others have previously shown that programmed death receptor-1 (PD-1) upregulation is a hallmark of tumour infiltrating, adoptively transferred T cells. PD-1 and its ligand (PD-L1) constitute a major immunosuppressive axis driven by tumour cells. Disruption of this axis may hit an Achilles heel of tumour immune escape.

Material and methods: A PD1-CD28 chimeric receptor was cloned into the retroviral vector pMP71 and expressed in primary murine T cells specific for the model antigen ovalbumin (OT-1 cells). Functionality was addressed *in vitro* using ELISA and flow cytometry. *In vivo*, ovalbumin and PD-L1 overexpressing Panc02 cells (syngeneic pancreatic cancer cell line) were inoculated subcutaneously in immunocompetent female C57Bl/6 mice. Mice (n = 6 per group) were treated twice i.v. with PD1-CD28 chimeric receptor-transduced T cells or control T cells.

Results: *In vitro*, PD-1-CD28 chimeric receptor-transduced primary T cells released 130 fold more interleukin-2 (IL-2) and 300 fold more interferon-γ than untransduced or control-transduced T cells when stimulated with CD3 and PD-L1, demonstrating the functionality of the chimeric receptor ($p = 0.0014$). In co-culture experiments with the Panc02 tumour cells, effective co-stimulation through PD1-CD28 was only seen in the presence of the TCR-recognized antigen ovalbumine and PD-L1. Upon blockade of MHC or PD-1, co-stimulation through the receptor was abrogated. Culture of transduced T cells in the presence of CD3 and PD-L1 increased cell numbers 4 fold and significantly increased viability of cells compared to untransduced or control-transduced T cells ($p < 0.0001$). *In vivo*, treatment of mice with an established (OVA and PD-L1 expressing) Panc02 subcutaneous tumour (mean tumour size at treatment onset 26 mm^2) with PD1-CD28-transduced OT-1 slowed tumour growth compared to treatment with control-transduced OT-1 cells ($p < 0.001$). This demonstrates the functionality of the chimeric receptor in an immunocompetent organism.

Conclusions: Adoptive T cells therapy with PD-1-CD28 chimeric receptor-transduced T cells is a promising approach to overcome PD-1-PD-L1-mediated tumour-induced anergy and immunosuppression.

I20

S82. Proffered paper: In-vivo testing of PSMA-targeted T-cell immunotherapy for prostate cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I20

Introduction: Bone is the most common site for metastasis in human prostate cancer patients. Skeletal metastases are a significant cause of morbidity and mortality and overall greatly affect the quality of life of prostate cancer patients. Despite advances in our understanding of the biology of primary prostate tumours, our knowledge of how and why secondary tumours derived from prostate cancer cells preferentially localise in bone remains limited. Examining the impact of these facets of bone metastasis in vivo remains a significant challenge, as animal models that closely mimic the natural history and malignant progression of clinical prostate cancer are not available.

Objectives: To develop an animal model of human metastatic prostate cancer. Once a model has been developed and optimised, it was this to test efficacy of immunotherapy using T-cells that have been genetically targeted against prostate-specific membrane antigen (PSMA).

Material and methods: Using PCR, western blot, flow cytometry and ELISA, we performed functional anaylsis of fucosyltransferase 3(FT3) in PC3LN3(PL)and PC3LN3-PSMA (PLP) tumour cell lines. In *vivo* bioluminescent imaging (BLI) was used to detect metastases.

Results: In preliminary studies, we have observed that delivery of a FT3-encoding retroviral vector to PL and PLP enables them to express sialyl Lewis X and to acquire E-selectin binding activity. We also showed that FT3 promotes increased PLP motility and invasiveness *in vitro*. Bioluminescent animal model of metastasised prostate cancer is established to determine the effect of this upon their pattern of metastatic spread in SCID Beige mice.

Conclusion: We have established an *in-vivo* model of PSMA-expressing prostate cancer. This will serve as a platform to test immunotherapy using P28z+ T-cells.

I21

S49. Clinical activity and development of biomarkers for an engineered anti PDL1 antibody MPDL3280A

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**I21

Background: Human cancer cells may suppress the adaptive immune response by expressing PD-L1 and down-regulating T cell activity through PD-L1/PD-1 and PD-L1/B7.1 interactions. Disruption of PD-L1 signaling restores anti-tumor immunity, resulting in durable responses across multiple human tumor types. Here we describe the clinical activity and development of predictive biomarkers for MPDL3280A, a human monoclonal antibody with an engineered Fc-domain designed to optimize safety and efficacy, that targets PD-L1 and prevents binding to receptors PD-1 and B7.1.

Materials & methods: We evaluated a multitude of biomarkers from pretreatment tumor specimens collected during clinical study of MPDL3280A. MPDL3280A has been administered as a monotherapy in over 300 pts with locally advanced or metastatic solid tumors in a phase 1 study.

Results: To date, MPDL3280A has been well tolerated across multiple dose levels and no G3-5 pneumonitis has been reported. Responses were observed in multiple tumor types, including NSCLC, RCC, and melanoma, with ongoing responses seen in the majority of responders. We report the association between pretreatment immune-related markers and response to MPDL3280A.

Conclusion: Overall, our assessment of tumor specimens not only has resulted in markers that may potentially identify response to MPDL3280A but also has furthered our understanding of the biologic activity of PD-L1 inhibition on treatment.

POSTER PRESENTATIONS

MONITORING OF IMMUNOTHERAPY

P1

P10. Concomitant gemcitabine therapy negatively affects DC vaccine-induced CD8+ T cell and B cell responses but improves clinical efficacy in a murine pancreatic carcinoma model

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P1

Background: Multiple studies have shown that dendritic cell (DC)-based vaccines can induce antitumor immunity. Previously, we reported that gemcitabine enhances the efficacy of DC vaccination in a mouse model of pancreatic carcinoma. The present study aimed at investigating the influence of gemcitabine on vaccine-induced anti-tumoral immune responses in a syngeneic pancreatic cancer model.

Material and methods: Subcutaneous or orthotopic pancreatic tumours were induced in C57BL/6 mice using Panc02 cells expressing the model antigen OVA (PancOVA). Bone marrow-derived DC were loaded with soluble OVA protein (OVA-DC). Animals received gemcitabine twice weekly. OVA-specific $CD8^+$ T cells and antibody titers were monitored by FACS analysis and ELISA, respectively.

Results: Gemcitabine enhanced clinical efficacy of the OVA-DC vaccine. Interestingly, gemcitabine significantly suppressed the vaccine-induced frequency of antigen-specific $CD8^+$ T cells and antibody titers. DC migration to draining lymph nodes and antigen cross-presentation were unaffected. Despite reduced numbers of tumour-reactive T cells in peripheral blood, *in vivo* cytotoxicity assays revealed that CTL-mediated killing was preserved. *In vitro* assays revealed sensitization of tumour cells to CTL-mediated lysis by gemcitabine. In addition, gemcitabine facilitated recruitment of $CD8^+$ T cells into tumors in DC-vaccinated mice. T and B cell suppression by

gemcitabine could be avoided by starting chemotherapy after two cycles of DC vaccination.
Conclusions: Gemcitabine enhances therapeutic efficacy of DC vaccination despite its negative influence on vaccine-induced T cell proliferation. Quantitative analysis of tumour-reactive T cells in peripheral blood may thus not predict vaccination success in the setting of concomitant chemotherapy.

P2

P11. Developing an immunotherapy strategy for the effective treatment of patients with Non Small Cell Lung Cancer (NSCLC): strategies to evaluate immunity in patients on clinical trials

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P2

Background: Our centers have formed an international collaborative group composed of surgeons, pathologists, radiation oncologists, medical oncologists and immunologists working together with biotech and pharma sectors to evaluate biomarkers of immune status and develop effective combination immunotherapy for patients with NSCLC.
Material and methods: Two phase I immunotherapies for NSCLC following surgical resection were begun at our institutions. One involved stage I-IIIA patients given an irradiated, autologous whole-tumour cell vaccine following induction of lymphopenia by chemotherapy and reinfusion of autologous peripheral blood mononuclear cells (PBMC). The other recruited stage IV patients given an autophagosome-enriched vaccine generated from irradiated autologous tumour cells. Histologic sections, enzymatically-digested tumour, pleural effusions and apheresis are available from a number of these patients. Current efforts are evaluating immunoscore and immune profiling by IHC and comparing results with flow cytometric analyses of the tumour.
Results: Preliminary studies have evaluated CD3+ CD8+ CD45RO+ cytotoxic memory T cells associated with tumour. We are continuing to examine myeloid to lymphoid infiltrate ratios. While preliminary, and with only 4 patients evaluated, preexisting autologous tumour-reactive T cells (IFN-γ) were only detected when T cells predominated in the tumour preparation analysed. Studies comparing this with IHC staining and analysis using Definiens software are underway.
Conclusions: A consortium of institutions has come together to improve the outcome of patients with NSCLC. The progress made to date will be used to evaluate immune responses to next generation immunotherapy. Evaluation of gene expression profiling of NSCLC has identified common overexpressed antigens and an off the shelf vaccine of autophagosomes containing at least 9 NCI prioritised cancer antigens, 5 TLR agonists and a DC targeting molecule has been developed. A multi-center phase II trial of combination immunotherapy for NSCLC employing this vaccine is open in the USA and efforts are underway to open this trial in Munich with coordinated efforts to evaluate anti-cancer immunity in patients on this and other trials.

P3

P12. Combination of radiotherapy and chemotherapy with dendritic cell immunotherapy in glioblastoma patients induces NK and NKT cell responses

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P3

Background: Two clinical studies including the treatment of first diagnosis and recurrent glioblastoma (GB) patients with dendritic cells (DCs) loaded with autologous tumour lysate (Nava et al, 2012) are currently active at Istituto Neurologico Besta, Milan.
Our first results obtained on a group of recurrent GB patients demonstrated that the response of NK cells correlates with a significantly prolonged survival (Pellegatta et al, 2013).
Material and methods: We have examined 17 patients with primary GB receiving standard radio and chemotherapy with temozolomide (RT-TMZ) after leukapheresis and TMZ in combination with DC vaccines. The median age at surgery was 57 y (range: 23–70). Peripheral Blood Lymphocytes (PBLs) were analysed by flow cytometry to identify CD8+ T cells, NK and NKT cells before and after DC vaccines. The ratio of vaccination/baseline frequencies (V/B ratio) of all of the immunological parameters for each patient was calculated, and the median of all of the observations used as the cut off value to separate patients into the 'low' or 'high' groups.
Results: RT-TMZ induced significant lymphopenia (<1000 lymphocytes/microl)) in 13/17 patients (76.4%). V/B ratio was correlated with the progression free survival (PFS) of each patient. Increased V/B ratio for NK cells and in particular NKT cells, but not for CD8 T lymphocytes, was significantly associated with prolonged PFS (median PFS 15 vs 8.5 mo, p=0.03; 15.0 vs 8.0 mo, p=0.002, respectively). Responder patients (PFS ≥ 12) showed increased expression of interferon (IFN)-γ immediately after the second vaccination as evaluated by real time-PCR.
No changes in the expression levels of IFN-γ were observed in the other patients.
Conclusions: Our results encourage further investigation on the role of NK and NKT cells in anti-tumour responses and on possible interference of radio-chemotherapy on activation of CD8+ T cells.

P4

P13. Intra-tumoral and surrogate immune responses in patients treated with the engineered anti-PD-L1 antibody (MPDL3280A)

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P4

Background: PD-L1 regulates CD8 T cell/Th1 immune responses. PD-L1 expressed in the tumor microenvironment can bind to PD-1 or B7.1 on activated T cells and mediate cancer immune evasion. MPDL3280A is a human mAb containing an engineered Fc-domain designed to optimize efficacy and safety that targets PD-L1 and blocks it from binding to its receptors.
Methods: Immunologic pharmacodynamics effects were evaluated in tumors and bloods from patients treated with MPDL3280A. MPDL3280A was administered IV q3w in >300 pts with locally advanced or metastatic solid tumors. PD-L1 and CD8 were measured by IHC. PD-L1 expression was evaluated in tumor and intra-tumoral immune cells. CD8 was assessed in the tumor center, periphery and invasive margin. The expression of ≈90 immune-related markers was evaluated at baseline (BL) and on-treatment using a custom-designed immunochip. BL tumor samples were available for 125 pts, and matched on-treatment samples were available for 31 pts. Further, blood-based biomarkers and circulating immune subsets were serially measured in 114 patients by modified ELISA and FACS, respectively.
Results: On treatment, responding tumors showed increase in expression of tumor cell PD-L1 and infiltration of CD8+ T-cells and a Th1-dominant immune infiltrate, providing evidence for adaptive PD-L1 up-regulation. Non-responders showed minimal tumor CD8+ T-cell infiltration and an absence of T-cell activation (measured by Granzymes, Perforin and EOMES expression). We also profiled circulating biomarkers for their association with clinical outcomes. A sub-population of patients, including those with RCC and melanoma, exhibited elevated PD-L1 expression on circulating T-cells at BL that was associated with response to MPDL3280A. On treatment, we observed a delayed increase in frequency of CD4+ICOS+ and CD4+PD1+ T-cells in

patients responding to MPDL3280A monotherapy. In contrast, frequency of CD8+HLA-DR+Ki67+ T-cells increased shortly following the first dose of MPDL3280A and returned to baseline levels by the end of cycle 2 when assessed in all patients, representing a transient pharmacodynamic measurement of PD-L1 inhibition. On-treatment, increase in plasma IL-6 was associated with disease progression. Associations with other circulating markers of inflammation including CRP with clinical outcomes will be presented.

Conclusions: Our data show that changes in pharmacodynamic immune markers are associated with clinical outcomes to MPDL3280A. These data provide mechanistic insights into immune checkpoint inhibition in cancer and identify potential biomarkers that may be monitored as on-treatment markers of clinical activity for patients treated with inhibitors of PD-L1/PD1 pathway.

IMMUNOMODULATION

P5

P14. Cell-based gene delivery leverages conventional immunotherapy for cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P5

Current immunotherapies against cancer comprise of target specific antibodies, cellular therapies like activated immune cells (e.g. DCs or NK cells), vaccination strategies, the use of tumour-specific infectious agents like oncolytic viruses or a combination thereof including conventional chemotherapies. All of those approaches attempt to target cancer specific pathways or certain immune response mechanisms, which have to be activated or at least present in the individual patient. This requires the identification of predictive biomarker and / or the execution of large clinical trials. Recent reports have shown that most clinical responses to targeted therapies are still quite unpredictable and yield in large clinical trials to demonstrate any benefit over conventional therapies.

Therefore there is still an unmet medical need to develop new therapies, identify novel target structures in cancer or combine different therapies to optimise the patient's outcome. Cell-based therapies as a pharmaceutical delivery tool for drugs or genes are expected to improve clinical medicine and the inflammatory tumour microenvironment (iTME) could be a novel target. We have combined those to innovative approaches to use mesenchymal stem cells (MSCs) delivering a gene directly into the iTME and activated a cytotoxic prodrug such as Ganciclovir only in the context of the tumour or its metastasis. This increases the local anti-tumour efficacy and reduces unwanted off-target toxicities. A first clinical trial (TREAT-ME1) targeting adenocarcinomas of the GI tract is currently being conducted.

Although the idea to use cells as pharmaceutical compounds is not new, the clinical application of Cell Therapeutics represents a new challenge for clinical scientists and all pharmaceutical applicants with regard to the current regulatory requirements for manufacturing, quality control and clinical trial approval in many parts of the world. The guidelines and standards are currently harmonized within the EU / FDA area of responsibility.

P6

P15. A genetic mouse model to identify the role of the immune adapter protein MyD88 in colorectal cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P6

Background: Pattern recognition receptors from the Toll-like receptor (TLR) family are pivotal components of innate immunity, and have been shown to contribute to colon cancer formation. However, the molecular and cellular mechanisms underlying TLR-signaling in colon cancer remain unclear. The adapter protein *Myeloid-differentiation factor 88* (MyD88) is shared between several TLRs and the Interleukin-1 receptor family. MyD88-deficiency protects mice from intestinal cancer formation in genetic models for colon cancer. The genetic mouse model presented here allows tissue-specific expression of MyD88, and thereby the dissection of the complex interaction between tumour and immune system during intestinal carcinogenesis.

Material and methods: Insertion of an 'intron-gene-trap' flanked with loxP motifs into the first intron of the MyD88 gene locus leads to global inactivation of *myD88* expression ($MyD88^{LSL}$), faithfully phenotyping a global gene knock-out. Tissue-specific re-expression of MyD88 in mice is mediated based on the Cre-recombinase. Breeding of $MyD88^{LSL}$ mice with LysMCre or pvillin-Cre mice leads to tissue-specific excision of the 'intron-gene-trap', retaining endogenous regulation of gene expression. MyD88 expression and successful reconstitution of TLR-signaling was detected in either myeloid cells ($MyD88^{MYEL}$) or intestinal epithelial cells ($MyD88^{IEC}$). Subsequently, these animals were mated with $Apc^{1638N/+}$ mice, an established genetic mouse model for human colon cancer.

Results: Global MyD88 deficiency dramatically decreased tumour incidence and aggressiveness in $Apc^{1638N/+}$ mice. Re-expression of MyD88 in intestinal epithelial cells only partially restored tumor formation. On the other hand, reconstitution of MyD88 expression in myeloid cells triggered tumour development virtually indistinguishable from parental $Apc^{1638N/+}$ mice. Activation of the canonical Wnt signaling pathway, induced by loss of function of Apc, was independent of MyD88. In contrast, MyD88 expression was required for full activation of MAPK/ERK signaling in intestinal epithelial cells. Furthermore, our results suggest a pro-tumorigenic function for the pro-inflammatory cytokines IL-1beta and IL-6, which were produced in a MyD88-dependent fashion by myeloid cells.

Conclusions: MyD88-mediated signaling has pro-tumorigenic effects in both IECs and in myeloid cells, but via different mechanisms. Moreover, MyD88 function in myeloid cells is crucial for intestinal tumour development, and its inhibition may form a promising therapeutic strategy.

P7

P16. Differential susceptibility of human and mouse NK cells to malignant cell-induced abnormalities in autologous combinations: a potential mechanism for the NK cell-based immunotherapy efficacy

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P7

Background: Natural killer (NK) cells are highly effective in controlling tumour growth, in mice, but have no significant effect in humans. The reason(s) of this phenomenon is(are) unclear.

Methods: The effects of cancer cells on NK cells during target-effector cell conjugation was investigated utilising standard immunological methods including flow cytometry, chromium release and enzyme-linked immunosorbent assays while gene expression was evaluated by quantitative reverse transcriptase-polymerase chain reaction.

Results: We found that this phenomenon was associated with the different susceptibility of human and mouse NK cells to autologous tumour cell-induced NK cell abnormalities (NKCA). The latter includes CD16 down-regulation and NK cell depletion. Induction of NKCA by leukaemia and solid tumour cells was influenced neither by IL2 treatment nor by HLA class I antigen expression, but was abrogated by a 10 day culture. Following a 10 day of PBMCs culture, NK cells became resistant to leukaemia and solid tumor cell induced NKCA but maintained their cytotoxic activity. Actinomycin D restored the susceptibility of long term NK (LTNK) cells to NKCA suggesting that the generation of resistance to NKCA required RNA transcription. TAPI-0, a functional analogue of the tissue inhibitor of metalloproteinases (TIMP) 3 inhibited cancer cell induced NKCA underlying a role for a restricted number of metalloproteinases in the generation of this phenomenon. Finally, we found an association of TIMP3 gene and protein over-expression with the reduced susceptibility of LTNK cells to cancer cell induced NKCA.

Conclusions: This study provides evidence that TIMP3 plays a role in the protection of LTNK cells from cancer cell induced NKCA.

P8

P17. RIG-I-like helicases induce immunogenic cell death of pancreatic cancer cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P8

Background: We recently identified RIG-I-like helicases (RLH) as therapeutic targets of pancreatic cancer for counteracting immunosuppressive mechanisms and apoptosis induction. Here, we investigated immunogenic consequences of RLH-induced tumour cell death.
Material and methods: Murine pancreatic cancer cells (Panc02) were treated with RLH ligands to induce apoptosis and were then cocultured with primary dendritic cells (DC). DC maturation marker expression, antigen uptake and antigen cross-presentation were assessed.
Results: RLH ligands induced production of type I IFN, HMGB1 and Hsp70 and translocation of calreticulin to the outer cell membrane of tumour cells. In cocultures, DC upregulated B7 expression, which was mediated by tumour-derived type I IFN, whereas TLR, RAGE or inflammasome signaling was dispensable. $CD8a^+$ DC effectively engulfed apoptotic tumour material and cross-presented tumour-associated antigen to naïve $CD8^+$ T cells. In comparison, tumour cell death mediated by oxaliplatin, staurosporine or mechanical disruption failed to induce DC activation, antigen uptake or cross-presentation. Moreover, tumour cells treated with sublethal doses of RLH ligands upregulated MHC-I and Fas expression and were sensitised towards CTL- and Fas-mediated killing.
Conclusions: RLH ligands induce a highly immunogenic form of tumour cell death linking innate and adaptive immunity.

P9

P18. The tumour leukocyte infiltrate is the key predictor for therapeutic response to catumaxomab therapy

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P9

Background: Patient stratification for therapeutic response mainly focus on biomarker expression on cancer cells. Contrary, the tumour micromileu is less considered, although stromal cells and leukocytes play a crucial role in tumour progression and drug resistance. The present study investigates the impact of tumour infiltrating lymphocytes on catumaxomab efficacy in CRC.
Patients and methods: Fresh tumour samples from 27 CRC patients were used to prepare tumour spheroids. Autologous PBMCs were isolated by ficoll density gradient. Tumour spheroids co-cultured with or without PBMCs were treated with catumaxomab for 96h. The cellular composition of the spheroids and the therapeutic impact on epithelial cells and leukocytes were measured by FACS analysis. The metabolic activity of the co-cultures was determined by the ATP assay.
Results: Similar to their original cancers all spheroid models consisted of a sufficient high fraction of EpCAM positive tumour cells indicating CRC an appropriate cancer type for catumaxomab therapy. In contrast, the fraction of CD45 positive leukocytes in spheroids was low mimicking the parental tumours. The effector cell to target cell (E:T, CD45 : EpCAM) ratio ranged from 0,05:1 to 1,92:1. Treatment of these spheroids with catumaxomab revealed no significant therapeutic effect. Addition of patient specific PBMCs changed the E:T ratio up to 7:1 and had a significant impact on antibody efficacy. Catumaxomab induced cell death was found up to 52% (p=0,015) depending on the individual E:T ratio. After catumaxomab treatment co-cultures demonstrated metabolic stimulation underlining the functionality of the leukocytes.
Conclusions: A dual biomarker system is required to select appropriate CRC patients for catumaxomab treatment. EpCAM expression on cancer cells is required, but the leukocyte infiltrate is the key predictor for the therapeutic response of catumaxomab. Both extent and functionality of the tumour infiltrating leukocytes have to be determined in the individual tumour sample for targeted therapy using the trifunctional monoclonal antibody.

P10

P19. Immunomodulation of blasts in AML-patients (pts) with clinically approved response modifiers to improve anti leukaemic T-cell reactivity: an ex vivo simulation of the clinical situation

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P10

Allogenic SCT/DLI are promising T-cell based therapies to cure AML-pts. Antileukaemic T-cell-reactivity has to be improved/re-established in pts in vivo. Ex vivo leukaemia-derived DC (DC_{leu}) are the most effective antileukaemic T-cell-stimulators.
Aim and methods: We generated DC_{leu} ex vivo from AML blasts from heparinised whole blood (**'WB-DC',** to simulate the in vivo situation) from 65 AML-pts in active stages of the disease using standard methods ('Picibanil', 'MCM-Mimic', 'Ca-ionophore', 'IFNa') or 11 minimalised cocktails (**'WB-minicock-DC',** combinations of 1-3 selected cytokines, antibiotics, bacterial lysates, or other clinically approved response-modifiers) and to correlate proportions of DC- or T cellsubsets and cytokine profiles with results with their ex vivo stimulatory capacity for antileukaemic T-cells and the pts' response to immunotherapy (SCT/DLI).
Results: 1. Generation of DC: we could identify 4 of 11 **mini-cocks**, that allowed the generation of DC/DC_{leu} from blast-containing WB-samples with at least one of the three methods. Some of the cocktails induced ex vivo blast-proliferation in individual pts. Proportions of DC-subtypes (e.g DC/DC_{leu}/mature DC) were comparable to proportions generated with standard DC methods. **2. Antileukaemic functionality:** In 21 cases T-cells stimulated with 1 to 3 '**WB-minicock-DC**' resulted in 56% cases with blast-lysis; in 6 pts 2-3 cocktails could be studied in parallel and in at least one of the cocktails a blastlysis could be achieved. Blast lysis (vs non-lysis) correlated with higher proportions of DC-subtypes: (DC,DC_{leu} blastconversion to DC_{leu}), higher proportions of T-cell-subtypes (viable, CD8 Tcells), higher concentrations of IL-12 and IFNg but lower concentrations of IL-6 and IL-8 **3.Clinical correlation**: AML-pts successfully responding to immunotherapy (SCT or DLI therapy) presented with higher proportions of DC, DC_{leu} and $CCR7^+$mature DC compared to pts without successful immunotherapy.
Conclusion: DC/DC_{leu} can be generated regularly from MNC or WB and with at least 1 to 4 of 11 minicocks containing combinations of 1-3 selected, clinically approved response modifiers. T-cells stimulated with 'WB-minicock-DC' achieved antileukaemic function, although not with every cocktail. A patient-individual testing of the best cocktail as well as the achieved antileukaemic (ex vivo) function can contribute to define cocktails of responsemodifieres to be applicated to AML pts to achieve or sustain remission.

P11

P20. Lack of T cell exhaustion in acute myeloid leukaemia

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P11

The prognosis of acute myeloid leukemia (AML), particularly when associated with adverse chromosomal or molecular aberrations, is poor due to a high relapse rate after induction chemotherapy. Postremission therapy for elimination of minimal residual disease remains a major challenge. Immunotherapeutic strategies aim at the stimulation of AML-specific immunity, especially of $CD8^+$ T cells. However, the functionality of these cells in AML patients is not well described. T cell exhaustion has been suggested to contribute to immune evasion in various solid and haematological malignancies. Primarily demonstrated in chronic viral infections, exhausted

T cells are characterised by an increased expression of several inhibitory molecules, reduced proliferation and an impaired capability of cytokine secretion and cytotoxicity.
To characterise T cell exhaustion in AML, we assessed the phenotype and effector function of $CD8^+$ and $CD4^+$ T cells by flow cytometry. T cells from patients at primary diagnosis, with refractory disease, at relapse and at relapse after allogeneic stem cell transplantation (alloSCT) were analysed for surface expression of CD244, CD160, PD-1, TIM-3 and LAG-3. T cell proliferation and production of the cytokines IFN-γ, TNF-α and IL-2 were measured in response to different stimuli. Results were compared to healthy controls (HCs), while untreated HIV-infected patients served as positive controls for an exhausted T cell state.
In HIV-infected patients, we observed a pronounced upregulation of the inhibitory molecules CD244, CD160 and PD-1 on $CD4^+$ and $CD8^+$ T cells as well as globally impaired cytokine production, clearly indicating T cell exhaustion. In contrast, T cells from AML patients showed an expression pattern of inhibitory surface molecules that was similar to T cells from age-matched HCs. AML patients with a relapse after alloSCT, however, showed remarkably high PD-1 expression on $CD4^+$ and $CD8^+$ T cells, accompanied by a shift from naive to memory T cells. Functionally, no defect in T cell proliferation in any of the AML patient cohorts was detected. Of note, however, we observed a 2-fold decrease in IFN-γ production by $CD4^+$ T cells exclusively in patients at primary diagnosis.
Thus, T cells of AML patients are fully functional. Immunotherapies that aim at eliciting tumour-specific immune responses, e.g. dendritic cell based vaccines, may therefore be particularly suited for AML treatment.

P12

P21. NK-cell dysfunction in human renal carcinoma reveals diacylglycerol kinase as key regulator and target for therapeutic intervention

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P12

NK cells are appreciated as antitumour effector cells in mouse models and human hematologic malignancies but their relevance in immunosurveillance of human solid tumours remains conflicting due to problems with *in situ* detection and reports of functional inactivity in the tumour milieu. The study was performed to identify mechanisms that impair NK-cell function in the tumour milieu and thereby identify therapeutic targets that allow recovery of NK-cell functionality.
We used *in situ* detection and flow cytometry to localise, quantify and profile NK cells of human clear cell renal cell carcinoma (ccRCC) tissue. Strategies were evaluated to reinstate functionality of tumour-derived NK cells. *In vitro* coculture models were applied to gain mechanistic insight into tumour-induced NK-cell alterations.
Tumour-resident NK cells, compared to NK cells from non-tumour kidney and PBLs, displayed conjoint phenotypic alterations and dysfunction induced by the tumour milieu, which were associated mechanistically with high levels of signaling attenuator diacylglycerol kinase (DGK)-a and blunted mitogen-activated protein kinase pathway activation (ERK1/2, JNK). Reinstating NK-cell functionality was possible by DGK-inhibition or brief IL-2-culture, interventions that de-repressed the ERK pathway. The extent of alteration and magnitude of recovery could be linked to NK-cell frequency within ccRCC-infiltrating lymphocytes, possibly explaining the observed survival benefit of patients with NK^{high} tumours.
DGK-mediated dampening of the ERK pathway ensuing in NK-cell dysfunction was identified as an important escape mechanism in ccRCC. DGK and the ERK pathway emerge as promising therapeutic targets to restore suppressed NK-cell activity for the improvement of antitumour immunity.

P13

P22. Immunomodulation induced by resveratrol or genistein on proliferation and apoptosis of tumour colon cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P13

Colon cancer represents a malignancy with a high incidence and mortality throughout the world, its etiology involving many genetic, immunological and biochemical factors. Anti-cancer drugs might exert their action during physiological pathways of apoptosis, leading to tumour cell destruction. One of the most effective anti-cancer agents used in the treatment of colorectal cancers is 5-fluorouracyl (5-FU), but tumour chemoresistance is a major limiting factor of its use. The purpose of this study was to assess whether different doses of plant bioactive compounds as genistein (GST) or resveratrol (RSV) might increase the anti-carcinogenic and anti-proliferative effects of 5-FU treatment and modulate gene *vs.* antigen expression of molecules involved in cell proliferation and apoptosis (P53, Bcl-2, Bax, Mdm2, PTEN) in colon tumour cell lines (LoVo).
The compound-mediated cytotoxity was measured by real-time cell analysis (RTCA) using xCELLigence System, a cell-based label-free platform technology that utilises the inherent morphological and adhesive characteristics of the cell. DNA progression through cell cycle phases was estimated by using PI staining (BD Cycletest Plus/DNA Reagent kit), while apoptosis was assessed by Annexin-V/FITC and PI double staining. In addition, antigen expression was evaluated by indirect immunofluorescence followed by flow-cytometry acquisition and analysis by FACS Canto II flow-cytometer. Gene expression was measured by qRT-PCR using Applied Biosystems® 7300 System.
Both GST and RSV single treatments, or combined with 5-FU induced a decrease of S% cell cycle phase, and an increse of apoptotic events. Different concentrations of GST or RSV blocked the treated cells in G2M; cells treated with RSV and 5-FU were blocked in G0G1 phase, while combined treatments with GST and 5-FU blocked the cells in G2M phase. The gene and antigen expression of molecules associated to cell proliferation and apoptosis were modulated by single and combined treatments, and additive effects of RSV or GST to 5-FU treatment were observed.
The chemo-preventive efficacy has been associated to enhanced apoptosis, therefore any therapeutic strategy that specifically triggers apoptosis in cancer cells might have potential immunotherapeutic value. By combining flavonoids with anti-cancer drugs, an increase of the antitumoral effects might be achieved, specifically in highly invasive cancer cells, while in non-tumoral cells the cytotoxic side effects could be reduced.

P14

P23. Efficient ex vivo lysis of acute myeloid leukaemic (AML) cells mediated by triplebodies with dual-targeting capability in conjunction with natural killer cells as effectors

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P14

Background: Current chemotherapy of acute myeloid leukaemia (AML) is limited by systemic toxicity. New agents are needed with increased selectivity for malignant cells and reduced toxicity.
Materials and methods: Triplebodies are a new class of antibody derivatives developed by our team, which consist of 3 antigen-binding domains carried in a single polypeptide chain. The distal domains bind 2 targets on the same cancer cell, the central domain binds a trigger molecule on an effector cell. If the 2 targets on the cancer cell are different, the triplebody is said to be 'dual-targeting'. The key new capability resulting from dual-targeting is the elimination of cancer cells with 'increased selectivity' (Schubert 2013; MAbs. PMID 24135631).
Here the dual-targeting triplebody 33-16-123 (SPM-2) was developed and tested in redirected lysis assays *in vitro*. It carries binding domains for the surface antigens CD33 and CD123 on AML cells and for CD16, the Fc gamma RIII receptor on Natural Killer (NK) cells. This pair of target antigens is expressed on the blasts of a majority of AML patients and is present in increased surface density on AML-leukaemia stem cells (LSCs) relative to bulk AML cells and healthy hematopoietic stem cells. This combination offers the possibility of preferential targeting of AML-LSCs, and thus of minimal residual disease (MRD) cells.
Human AML cell-lines and primary cells freshly drawn from AML patients were used in redirected lysis experiments. Target cells were labeled with Calcein AM. Effector cells were either *ex vivo* expanded mononuclear cells

from healthy donors, or patient's autologous NK cells enriched by immunomagnetic beads. Reactions proceeded for 4 hrs, and specific lysis was measured by release of fluorescent Calcein using an ELISA plate reader. Multicolor cytofluorimetry with fluorescent-labeled antibodies was used in addition to study elimination of subsets of AML cells.
Results: SPM-2 effectively eliminated human AML cell lines including MOLM-13, which are double-positive for CD33 and CD123, with an EC50 concentration in the range of 20-50 pM, as well as primary blasts from AML patients with various subtypes of AML with EC50 concentrations in the range of 50-100 pM. Less than 10% of myeloid cells from healthy donors enriched for CD33-positive cells were lysed by SPM-2 used in a 10 nM dose. Therefore, a clear therapeutic window appears to exist. For some patient samples SPM-2 also produced effective lysis of the CD34pos and the CD34pos CD38neg CD123pos subsets, which encompass the MRD cells.
Conclusions: SPM-2 mediated efficient redirected lysis *in vitro* by NK-cells, both of established human AML-derived cell lines and of freshly drawn blasts from patients with various subtypes of AML. SPM-2 also mediated lysis of cellular subsets encompassing the AML-LSCs and thus of the MRD cells responsible for relapsed disease. The agent is promising for lysis of AML cells *in vivo* with increased selectivity and reduced systemic toxicity and is scheduled for clinical development.

P15

P24. Aviscumine enhances NK- cytotoxicity against tumor cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P15

Background: The mistletoe lectin I belongs to a new class of anticancer drugs with type II ribosomal inhibitor activity. The recombinant mistletoe lectin (aviscumine) has shown immunomodulatory and cytotoxic activity in preclinical models as well as potential antitumor effects in phase I and I/II clinical trials. The aim of this study was to further elucidate the immunostimulatory capacity of aviscumine on natural killer (NK) cell function in a human ex-vivo model.
Methods: The effect of aviscumine (0.5 and 1 ng/ml) on the cellular cytotoxicity of NK cells isolated from peripheral blood mononuclear cells (PBMCs) of 34 healthy volunteers was measured via a standard Chromium^{51} release assay against K562 chronic myelogenous leukemia cells. For further validation changes in expression of the NK cell activation marker CD107α was determined via flow cytometry (FACS) in 13 volunteers.
Results: Aviscumine induced a significant concentration-dependent increase in NK cellular cytotoxicity in about 54% of the volunteers ($p<0.001$). This enhancement was also observed with low dose IL-2 stimulation ($p=0.01$). FACS analysis revealed an aviscumine triggered up-regulation of the NK cell degranulation marker CD107a ($p=0.001$).
Conclusion: Functional ex-vivo analysis of NK cells from healthy donors revealed a direct immune stimulatory mechanism of aviscumine. These data further strengthen its potential as immunomodulatory antitumor agent and suggest that NK cell activity in peripherial blood may be evaluated as predictive biomarker in clinical trials.

OTHER

P16

P25. Access to diagnostics: a bottleneck for immunotherapeutics development - case example of MAGE-A3 cancer immunotherapeutic

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P16

Precision medicine with modern immunotherapeutics often requires pre-therapeutic access to novel and sometimes complex diagnostic tests. Test development and access to tests including reimbursement often lacks behind medicine development and availability of medicines, potentially creating a gap for physicians to administer and patients to receive an optimised treatment regime. Pharmaceutical companies are challenged by the fact of having to co-develop a diagnostic method, a process which many of them are not accustomed to. In addition, many healthcare systems have no way of allowing in parallel evaluation of diagnostic test and medicine with a view to reimbursement.
This presentation illustrates approaches using the developmental history of the MAGE-A3 cancer immunotherapeutic to both address the developmental hurdle through innovative models and partnerships, as well as the market access hurdle through appropriate market understanding and evidence generation.
Insights and solutions of pre-clinical, clinical and market research/insight will be presented.

P17

P26. Cancer associated fibroblasts contribute to the immune suppression of breast cancer by augmentation of the inflammatory products

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P17

Background: The supporting stroma of breast cancer is believed to support the growth and metastasis of cancer cells and are responsible for suppressing anti-cancer immune responses. In this regard we attempted to isolate and characterise the cancer associated fibroblasts (CAFs) of murine model of spontaneously developed breast cancer.
Methods: CAFs were isolated by explants culture of tumour tissue. The Fibroblast activated protein-alpha(FAP-α)-positive fibroblasts were co-cultured with splenocytes where the splenocyte proliferation and production of inflammatory and regulatory cytokines were assessed by ELISA. Also the inflammatory enzymes iNOS and the production of matrix metaloproteinases 2 and 9 by these cells were evaluated using Real-Time PCR.
Results: Findings indicated enhanced in vitro immune suppression in co-cultures of CAF and splenocyte. Additionally, increased regulatory cytokine and inflammatory mediators was observed.
Conclusion: The secretory profile of these cells, as the supporting matrix, is a massive physical and immune barrier to anti-cancer immune therapy. Therefore it is proposed for enhancing the effect of therapy must take into acount the contribution of cancer associated fibroblasts on the chronic inflammatory microenvironment of breast cancer.

P18

P27. Alemtuzumab (anti-CD52 monoclonal antibody) as single-agent therapy in patients with relapsed/refractory chronic lymphocytic leukaemia (CLL) – a single region experience on consecutive patients

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P18

Alemtuzumab, a humanised CD52 monoclonal antibody, is routinely used as treatment for patients with refractory chronic lymphocytic leukaemia (CLL). Although alemtuzumab has been evaluated in numerous prospective clinical trials, little is known about its safety and efficacy in the routine clinical practice setting. Given that the haematology centers in the Stockholm area has gained substantial experience on alemtuzumab usage, starting already in the early 1990s, it would be of interest to compare our results obtained in routine health care with those from other reports including multicenter clinical trials.
Records from 1301 patients with CLL from the Stockholm-Cancer-Registry (1991-2010) identified 56 patients treated with alemtuzumab in the relapsed or refractory setting.
The median age was 69 years, 88% had advanced Rai stage with a median of 3 prior lines of therapy. One fourth had bulky lymphadenopathy and 73% were refractory to purine analogues. The median cumulative dose of alemtuzumab was 930 mg, being significantly higher ($p=0.0277$) for

responders (1063 mg) compared to non-responders (643 mg). The median duration of therapy for responders was 12.4 weeks (range 7-32 weeks) and in all patients 11.6 weeks (range 1-51 weeks). The overall response rate (ORR) was 43%, with a response rate of 32.5%, 50% and 87.5% in the refractory, purine analogue relapsed and non-purine analogue relapsed group respectively. The differences in response rate was statistically significant (p=0.0104). A good performance status (PS) was associated with better response rate (ECOG 0-1 vs. ≥2) (p=0.0227). The median time to treatment failure (TTTF) was 7.8 months (range 0.4-55.4 months) being significantly longer for responders, 13.4 months (range 3.9-55.4 months) than for non-responders, 6.1 months (range 0.9-16.3 months) (p<0.0001). The median time to next therapy was 12.7 months (range 0.4-55.4 months).
Major infections (defined as ≥ grade III) occurred in 36% of the patients. Cytomegalovirus reactivation was the most common opportunistic infection (75%) occurring in 9 patients. Median overall survival was 22.5 months (range 0.4-74.3 months). Responders had a significantly longer survival than non-responders, 44.2 and 16.3 months respectively (p=0.0006, log-rank test), although the difference when using the Landmark method was not significant. Predictive factors for longer survival was PS grade 0-1 (p<0.0001) and fewer previous treatment lines (1-3 vs. ≥4) (p=0.0038, log-rank test). Twelve patients were retreated with alemtuzumab at least once with an ORR of 50% and a TTTF of 7 months (range 0.5-13 months).
In summary, a high cumulative dose/longer duration of therapy as well as relatively high response rates and long median survival was observed compared to what has been reported earlier in similar groups of patients having received alemtuzumab in trials or in routine health care in other areas. Optimal patient identification and management may result in avoidance of early discontinuation and possible better treatment outcomes.

P19

P28. Efficient CD19-positive leukaemia cell lysis mediated by a T cell-recruiting triple body a[19-3-19]

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P19

Background: The design of antibody derivatives with higher tumour cell selectivity is hoped to improve cancer immunotherapy and to decrease side effects. One promising molecular format that we have developed is the triple body. A triple body allows for selective lysis based on its 'dual targeting' capacity. The triple body format consists of three linked single chain variable fragments (scFv) with the two distal scFvs targeting different antigens on the tumour cell surface. The central scFv triggers an available immune effector cell for redirected lysis of the tumour cell. For the triple body anti[HLA-DR-CD16-CD19], it was previously shown that double-positive target cells were preferentially eliminated by NK cells versus single-positive targets. In the present study, a triple body designated α[19-3-19] with specificity for CD19 and CD3ε was constructed, produced and tested to determine whether a triple body could also recruit effector T cells as this has not previously been demonstrated.
Materials and methods: For the construction and production of α[19-3-19], standard molecular biology procedures were employed. Flow cytometry was used to determine the specific binding behaviour of α[19-3-19] and to characterise various T cell subsets. The cytotoxic potential of α[19-3-19] was evaluated in redirected lysis assays using variable effector-to-target ratios against the B cell lines SEM, Nalm-6, Raji, Namalwa and ARH77 as well as cells from an MPAL patient.
Results: We show that the triple body α[19-3-19] binds to CD19-positive cells and primary T cells. When tested in cytotoxicity assays, the triple body mediated up to 95% specific lysis of CD19-positive tumour cells from established B-ALL cell lines as well as against cells isolated from primary patient material using pre-stimulated allogeneic effector T cells *in vitro* after 3 hours incubation. At effector-to-target ratios of 1:10, α[19-3-19] still led to the depletion of approximately 60% of CD19-positive cells. Furthermore, the α[19-3-19] triplebody activated resting T cells from healthy unrelated donors and induced the specific lysis of autologous CD19-positive cells.
Conclusion: These results show that the molecular format of the triple body using CD3ε as a T cell trigger is also suitable for the specific killing of leukaemia cells. In addition, the 'dual-targeting' capacity of triple bodies could make these molecules potentially powerful therapeutic agents for the selective and efficient elimination of leukaemia cells while sparing normal healthy cells.

P20

P29. T-cell responses to oncogenic Merkel cell polyomavirus proteins distinguish Merkel cell carcinoma patients from healthy donors

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P20

Purpose: Merkel cell carcinoma (MCC) is a highly aggressive skin cancer with strong evidence for viral carcinogenesis. The association of MCC with the Merkel cell polyomavirus (MCPyV) may explain the explicit immunogenicity of MCC. Indeed, MCPyV-encoded proteins are likely targets for cytotoxic immune responses to MCC as they are both foreign to the host and necessary to maintain the oncogenic phenotype. However, to date only a single MCPyV-derived CD8 T-cell epitope have been described, thus impeding specific monitoring of T-cell responses to MCC.
Method: To overcome this limitation, we scanned the MCPyV oncoproteins large T and small T antigen and the virus-capsid protein VP1 for potential T-cell epitopes, and tested for major histocompatibility complex (MHC) class I affinity. We confirmed the relevance of these epitopes using a high-throughput platform for T-cell enrichment and combinatorial encoding of MHC class I multimers.
Results: In peripheral blood from 38 MCC patients and 30 healthy donors we identified 53 MCPyV-directed CD8+ T-cell responses against 35 different peptide sequences. Strikingly, T-cell responses against oncoproteins were exclusively present in MCC patients, but not in healthy donors. We further demonstrate both the processing and presentation of the oncoprotein-derived epitopes, as well as the lytic activity of oncoprotein-specific T cells towards MHC-matched MCC cells. Demonstrating the presence of oncoprotein-specific T cells among tumour infiltrating lymphocytes *ex vivo* further substantiated the relevance of the identified epitopes.
Conclusion: These T-cell epitopes represent ideal targets for antigen specific immune therapy of MCC, and enables tracking and characterisation of MCPyV specific immune responses.

P21

P30. The use of HER2 receptors status as a prognostic index for estrogen receptor positive breast cancer patients

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P21

Background: Tamoxifen has been a standard adjuvant hormonal treatment for estrogen receptor ER positive breast cancer, both in pre and postmenopausal women. It has been noticed that some breast cancer patients don't respond to tamoxifen as others. In the presented study, HER2 receptor status has been studied as a probable prognostic index regarding the local recurrence & disease related mortality & to differentiate responders from non-responders to tamoxifen treatment.
Methods: 205 patients in the oncology center of Merjan Hospital, Hilla, Iraq who had mastectomy performed and histopathology done studying the ER and HER2 receptor status, all of which ER positive and of early stages (I and II) breast cancer, had been studied prospectively between the fifth of June 2010 and the 19th of June 2011 regarding the local recurrence and mortality during this period. All the patients had been given Tamoxifen tablet 20mg/day.
Results: 56 (27.3%) were HER2 receptors +ve, and 149 (72.7%) were HER2 receptor -ve . 19 (33.9%) and 9(16%) of the HER2 +ve patients had local recurrence and died due to the disease respectively during the follow up period. 30 (20.1%) and 14 (9.4%) of the HER -ve patients had local recurrence and died of the disease respectively during the follow up period.

Conclusion: I found a significant difference in the disease local recurrence and mortality of ER+ve HER2 receptor +ve as compared with the ER+ve HER2 receptor -ve in favor of the latter and the ER+veHER2 receptor -ve had a much better prognosis.

P22

P31. Genomic profiling and functional characterisation of a new myeloid cell type enriched in renal cell carcinoma

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P22

Dendritic cells (DCs) are central players of the immune response because they regulate both innate and adaptive immune cells. As part of the leukocyte infiltrate of various tumours they can initiate antitumour immune responses through the activation of tumour-specific T cells.
However, tumours employ strategies to evade the immune response by manipulating the differentiation and activation of DCs.
Previously we described 'enriched-in-renal-carcinoma DCs' (ercDCs) as an unusual $CD14^{+}CD209^{+}$ expressing myeloid cell type that displays characteristics of both DCs and macrophages. As one example, ercDCs showed good antigen cross presentation which is a prototypic DC function and a prerequisite for the activation of T effector cells in local tumour tissue. To better understand the relationship of ercDCs to other myeloid cell types and to get insight in functional pathways operative in ercDCs we performed transcriptome analysis using Affymetrix® gene arrays comparing ercDCs, cDCs, proinflammatory M1 and GM-CSF macrophages and alternatively activated M2 macrophages. Cell types were generated in parallel from monocytes of 15 healthy donors. RNA was extracted using the Qiagen® RNeasy Micro kit. To reduce population variability, RNA from each cell type- from 5 donors- was pooled and 3 biological replicates generated. Clustering of normalised values showed that ercDCs differ from the other myeloid cell types. Further analysis included heat mapping and creation of signaling pathways using pathvisio software. Focusing on the cross presentation pathway, a gene list was assembled through literature mining and each gene was assigned an expression value using microarray data. A comparison using the heat map representation revealed that the expression pattern of ercDCs was most similar to GM-CSF macrophages and least to M1 macrophages. Comparison based on fold changes generated by normalisation to cDCs revealed highest similarity between ercDCs and M2 macrophages. Particularly, transcription of genes involved in receptor-mediated endocytosis and the vacuolar pathway were upregulated in ercDCs indicative that cross presentation could be active in ercDCs.

P23

P32. High resolution mass spectrometry reveals the depth and diversity of HLA-I peptidomes

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P23

T-cell responses against infected and cancer cells are initiated by recognition of HLA-I peptides (the peptidome) presented on the surface of nucleated cells. The repertoire of HLA-I peptides originates primarily from sampling the cytosolic degradation products of intracellular proteins. HLA-I peptides have been extensively studied in the last years because of their immediate use as immunotherapy based cancer vaccines. Even more advanced cell based therapeutic applications are being developed based on cancer specific HLA-I peptides. In this study, we used high resolution mass spectrometry and the MaxQuant bioinformatics environment to obtain a high accuracy and in-depth coverage of HLA peptidomes. HLA-I peptidomes were immuno-affinity purified from 10 cancer and primary cell lines. The unprecedented high number of thousands of identified HLA-I peptides per cell line enabled us to evaluate the known mechanisms governing peptidome presentation and to determine the proteome which is sampled for presentation. We envision that applying our methodology for analysing patients tumour samples will result in the discovery of new cancer specific peptides. Better immunotherapeutic modules could possibly be developed based on wider and more accurate repertoires of HLA-I peptides and they will increase the accessibility of these therapies for a larger cohort of patients.

P24

P33. NK-92 cellular immunotherapy as an alternative to donor derived peripheral blood NK cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P24

Introduction: Infusions with cytokine-activated NK cells obtained from blood of MHC mismatched donors have shown some promising results especially in patients with myeloid leukaemia. There is also indirect evidence that infusions of NK cells as part of a stem cell transplant result in lower relapse rates post-transplant when donor and recipient are mismatched for KIR receptors on NK cells.
Methods: Collecting NK cells form donors requires them to undergo leukapheresis with subsequent removal of CD3+ lymphocytes (to prevent GvHD) and expansion and activation of the NK cell enriched fraction with IL-2. In contrast, the continuously growing NK cell line NK-92 can be easily expanded to clinical scale in bioreactors. The broad cytotoxicity of NK-92 is due to the lack of most of the KIR receptors while expressing a range of activating receptors.
Results: We report here results from concluded and ongoing clinical phase I studies with NK-92 cells in patients with advanced cancer, that confirm its safety profile. Anti-tumour responses were seen in patients with some advanced haematological malignancies and solid tumours. NK-92 also provides a platform for further genetical engineering. A variant has been generated that expresses a high affinity FcgIIIRA receptor that can augment the treatment efficacy of mAbs that utilise ADCC to kill target cells. Various investigators are using the NK-92 cells as a platform to introduce specific tumour antigen receptors (i.e. CAR) to make them targeted to specific tumors such as melanoma, myeloma, leukaemia or brain cancer. Video-lapse studies show that those CAR engineered NK-92 cells specifically kill tumour antigen expressing cancer cells and are able to do 'serial killing'. The cell expansion process for NK-92 has been streamlined and made more economical using various bioreactor designs.
Conclusion: The human clinical grade NK-92 cell line has many advantages over peripheral blood NK cells as a cell therapy product for cancer patients. Results from phase I clinical trials confirm its safety profile. This unique cell line is now tested in phase II studies for efficacy in various cancers and is also further developed through genetic engineering to target specific tumours through CAR. NK-92 are an off the shelf tumour targeted local and systemic cell treatment.

VACCINES

P25

P51. Identification of prostate cancer-associated antigens by oxygen manipulation

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P25

Background: Therapeutic vaccination against prostate cancer (CaP) remains marginally effective. A reason for failure may stem from the fact that vaccine cells are usually cultured in ordinary air. Solid tumours including CaP contain

regions with oxygen deficiency (hypoxia) secondary to the lack of blood supply to the growing tumour nodules, which may lead to changes in expression of cancer-associated antigens in tumour cells. To test this hypothesis we determined whether oxygen-sensitive CaP-associated antigens in cultured CaP cell lines and human tumour tissues exist.

Material and methods: LNCaP and VCaP prostate cancer cells were propagated in culture media conditioned by the cells at normoxic (20% O_2) and hypoxic (2% O_2) environment. At first, we measured release of vascular endothelial growth factor (VEGF) by ELISA and the expression of VEGF-α mRNA by RT-PCR. To identify potential CaP-associated antigens, we prepared CaP cell lysates, resolved them by 2D electrophoresis and immunoblotting using spontaneous antibodies from plasma derived from CaP patients and control subjects. Antibody-labelled spots were analysed by MALDI-TOF mass spectrometry. Furthermore, we evaluated the expression of selected candidates in native CaP tissue.

Results: Hypoxic CaP cells released more VEGF ($P<0.05$) and expressed more mRNA for VEGF-α ($p<0.001$) than normoxic cells. After two days of culture, hypoxic cells expressed some forty fold higher amount of VEGF transcripts compared to normoxic cells. CaP-associated spots identified in this study included heat shock protein 70 (HSP70), HSP60 and heterogeneous nuclear ribonucleoprotein L (hnRNP L). Among them, HSP70 and hnRNP L were O_2-sensitive. Level of the two proteins were two times higher in CaP tissue than in control benign prostate tissue ($p<0.05$).

Conclusion: A unique set of O_2-sensitive CaP-associated antigens exist in CaP tumour tissues and spontaneous antibodies are detected in plasma derived from CaP patients. Therefore, CaP cells grown at hypoxic condition may provide a better antigen match to tumours *in situ* and may be more effective vaccines. (Supported by UMMC Incentive (to TMA), DOD PC094680 and PCF Creativity Award (to CRG).

P26

P52. A new pathway of tumour antigen loading of human dendritic cells via intercellular communication

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P26

Background: Most cancer cells down-regulate gap junctions (GJ) resulting in loss of communication with their surrounding microenvironment. We have previously shown that infection of mouse tumour cell lines with *Salmonella* induces the up-regulation of connexin 43 (Cx43), a ubiquitous protein that forms GJs. This up-regulation allows the transfer of antigens between tumour cells and dendritic cells (DC) licensing them to induce an efficient anti-tumour response in a mouse model of melanoma (Saccheri et al., Sci TM 2010).

Herein, we tested the idea that through the formation of intercellular communication channels in tumour cells, human autologous dendritic cells could be loaded with tumour antigens, preprocessed by cancer cells, and could be used to generate a cancer vaccine.

Material and methods: Human melanoma cell lines were infected with a vaccine strain of *Salmonella Ty21a* (*Vivotif*) to induce up-regulation of Cx43 and formation of GJ channels. HLA matched DCs were differentiated in vitro by peripheral blood purified monocytes from healthy donors. Expression of surface molecules, cytokine release and cell proliferation was determined by cytofluorimetry. Cytotoxicity was determined either by Delfia cell cytotoxicity assay (PerkinElmer) and by a cytofluorimetry based CD107 assay.

Results: *Vivotif*-infected tumor cells established GJs in human melanoma cell lines. We show that tumour derived antigens could transit from 'donor' melanoma cells' cytoplasm to DC's cytoplasm through GJs, generate a tumour-specific CTL response and reactivate tumour infiltrating lymphocyte (TIL) in vitro. The transfer of tumor antigens was GJ-dependent because it was abolished in the presence of a GJ-specific inhibitor. In vitro generated tumour-specific human CTLs lysed also non-donor melanoma cell lines indicating that DCs were loaded with tumour-associated antigens shared among melanoma cell lines.

Moreover, in vitro generated CTLs were melanoma specific because they were unable to lyse HLA matched colorectal adenocarcinoma cell lines.

Conclusions: We exploited an antimicrobial response present in tumour cells to activate cytotoxic CD8 T cells specific for tumour-peptides, through a new pathway of antigen loading of human dendritic cells via intercellular communication channels. This unique and novel approach can be applied across a wide range of tumour cell types and can be used clinically as therapeutic vaccine alone or in combination with gold standard treatments.

P27

P53. Effect of lymphodepletion and tumour on host and reconstituted regulatory T-cells in a model of murine melanoma

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P27

Background: Activation of tumour-specific T-cells is enhanced during homeostatic proliferation of lymphocytes in mice receiving *l*ymphodepletion, immune *r*econstitution and *a*ctive-specific *t*umour cell vaccination (LRAST). Nevertheless, the induction and the therapeutic efficacy of the tumor-specific T-cells is attenuated by immunoregulatory mechanisms such as regulatory T cells (Treg). Following LRAST, Treg can be derived from the host or the donor lymphocyte pool. Aim of the following study was to track the origin of the immune modulating Treg (host vs. donor) and to analyze their fate following LRAST.

Material and methods: C57BL/6 mice were injected with D5 melanoma cells ($5x10^4$ s.c.) to generate tumour bearing mice. After 3 days cyclophosphamide (200mg/kg i.p.) was injected to induce lymphopenia. On day 1 after lymphodepletion, mice were reconstituted with $2x10^7$ naïve spleen cells from a congenic strain of mice (C57BL/6-Ly5.1, $2x10^7$ cells i.v.) and vaccinated with irradiated, GM-CSF-producing D5G6 cells ($1x10^7$ s.c.). These cells could be distinguished from the host T-cells based on their leukocyte surface molecule (host leukocytes: $CD45.2^+$, donor leukocytes: $CD45.1^+$). Mice without lymphodepletion served as a control. On day 3, 10 and 23 after lymphodepletion, cell populations were phenotypically analyzed by flow cytometry of splenocytes and blood cells. $CD3^+CD4^+CD25^+Foxp3^+$ cells were considered Treg; tumour specificity was controlled by IFN-g cytokine release assays.

Results: In the lymphodepleted mice, flow cytometric analysis of splenocytes showed a decline in the host Treg between day 3 and day 10, followed by a recovery of the cells until day 23. In mice without lymphodepletion, host Treg remained at a constant level throughout the experiment. In contrast to that, we observed a steady increase over time in reconstituted Treg with a 5- to 8-fold increase from day 3 to day 23 in both lymphodepleted and not lymphodepleted mice. In LRAST-treated mice we were able to induce a higher frequency of tumour-specific T-cells compared to not lymphodepleted mice, as observed by tumor-specific cytokine release assays.

Conclusion: Due to the proliferation of transferred Treg and increased levels of host Treg after lymphodepletion until day 23, the induction of tumor-specific T-cells might be limited. In future experiments we will determine whether the selective reduction of transferred Treg or the depletion of Treg with a specific anti-CD25 monoclonal antibody in the host will improve the induction of tumour-specific T cells and the therapeutic efficacy of active-specific tumour vaccination following LRAST.

P28

P54. Possible role of MDSC for the induction of tumour-specific T cells following LRAST

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P28

Background: *L*ymphodepletion with subsequent *r*econstitution and *a*ctive-specific *t*umour cell vaccination (LRAST) during the homeostatic proliferation is a promising approach in cancer immunotherapy. While tumour specific response is evident under LRAST regime, simultaneous induction of potentially immunosuppressive immature myeloid cells (MDSC) may limit the therapeutic efficacy. Following LRAST MDSC can potentially be derived from the host or the donor cell pool. Aim of the study was to analyse the role of granulocytic (G-MDSC: $CD11b^+$ $Ly6G^+$ $Ly6C^+$) and monocytic (Mo-MDSC:

$CD11b^+$ $Ly6G^-$ $Ly6C^+$) MDSC and to track their fate in the recipient mouse following LRAST.

Material and methods: Female C57BL/6 (Ly5.2) mice were challenged with tumour by subcutaneous injection of 5×10^4 vital D5 melanoma cells and lymphodepleted 3 days later by i.p. application of 200mg/kg cyclophosphamide. After 24 h mice were reconstituted with i.v. injection of 2×10^7 splenocytes from naïve B6.SJL-PtprcaPepcb/BoyCrl (Ly5.1) mice followed by vaccination with 1×10^7 irradiated mGM-CSF-producing D5G6 cells. Mice lacking tumour challenge, lymphodepletion, or tumour vaccination were included as controls. Splenocytes, peripheral blood cells and lymph node cells were analyzed by 8 color flow cytometry at days 3, 10 and 23. Induction of tumour-specific T cells in tumour vaccine draining lymph nodes was evaluated in vitro by tumour specific IFN-γ secretion assays.

Results: A pronounced increase in host G- and Mo-MDSC was observed in spleens of both lymphodepleted and vaccinated groups between day 3 and 10. A decrease of T-cell subsets, including memory T-cells, and natural killer (NK) cells was detected at day 10. Consecutively to the rapid decline of MDSC after day 10 a numeric gain in the T-cell fraction was observed. In contrast NK cells remained at a low level until day 23. Donor G- and Mo-MDSC were detected at low frequencies at all time points. Donor MDSC as identified by Ly5.1 slightly increased between day 3 and 10 followed by a decline after day 10. The reconstituted cell fraction predominantly consisted of T-cells (up to 29.7% of leukocytes). Helper and effector T-cells were continuously increasing during the observed period of time while memory type T-cells ($CD8^+$ $Gr1^+$) were being suppressed.

Conclusions: The elevated numbers of MDSC at day 10 after treatment may inhibit the induction of tumor specific T cells. Therefore depleting MDSC in the recipient mouse with anti-Gr1 antibody in the early phase of the LRAST treatment could be a promising approach to improve anti-tumour efficacy.

P29

P55. Dendritic cell vaccination for postremission therapy in AML

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P29

Cellular immunotherapy is a highly effective treatment option for patients with acute myeloid leukaemia (AML) as shown by the low relapse rate after allogeneic stem cell transplantation. However, many patients are not eligible for this treatment. This has lead to the development of various immunotherapeutic approaches that aim at inducing autologous cellular and humoral immune responses against AML and specifically against residual leukaemic stem cells (LSCs).

Dendritic cells (DCs) are important regulators of the human immune response. We have developed a three-day DC manufacturing protocol that starts with peripheral blood monocytes, e.g. from AML patients in remission following intensive chemotherapy. By using a cytokine cocktail containing a synthetic TLR7/8 agonist, the resulting DCs develop improved immunogenicity. For healthy donors as well as for AML patients, we were able to show that these DCs display a positive costimulatory profile, secrete high levels of IL-12p70, show chemotaxis to CCR7 ligands, and activate NK cells. After loading the DCs with mRNA, they effectively induce antigen-specific T cell responses with a strong type-1 polarization. Due to these properties, this DC type seems highly suitable for application in cancer immunotherapy.

We have recently initiated a phase I/II clinical trial for the application of these DCs in the setting of AML postremission strategy. WT1 and PRAME were chosen as leukaemia-associated antigens due to their overexpression on leukaemic blasts and specifically on cells that are enriched for LSCs. DCs transfected with mRNA encoding CMV-pp65 are included into the vaccine as an adjuvant as well as a surrogate antigen. 20 patients with a non-favourable risk profile or with confirmed minimal residual disease (MRD), but who are not eligible for allogeneic stem cell transplantation, will be included. The primary objective of this study is to evaluate feasibility and safety of this immunotherapeutic approach. Important secondary endpoints are immune responses to the applied antigens and MRD control. First results of this study will be presented.

P30

P56. A novel cancer vaccine with nanogel-based antigen transporter and sequence-optimised long peptide antigen

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P30

Background: The limited success of cancer vaccines in clinical could be owing to their low efficacy that can be largely affected by their formulation. Vaccine formulation usually consists of three components, antigen, delivery system, and adjuvant. Recently, long peptide antigen (LPA) has been attracting interest, because (1) one LPA can provide antigen presenting cells (APCs) with multiple T cell epitopes and (2) it avoids undesired presentation by non-professional APCs that can lead to the exhaustion of antigen-specific T cells. While a LPA can include multiple epitopes, the prerequisite design of junction of epitopes (inter-epitope sequence, IES) for robust immunogenicity is not yet established, and we explored it in this study. On the other hand, accumulating data has been suggesting the importance of delivery system for cancer vaccines. By employing a novel nanogel-based delivery system, we tried to improve the immunogenicity of LPA vaccine.

Materials and methods: LPA was designed to include three mouse cytotoxic T cell (CTL) epitopes that were linked with the IES of oligotyrosine, oligothreonine, oligoglycine or oligoproline. The complex of LPA and cholesteryl pullulan (CHP) nanogel was fabricated, and subcutaneously injected to mice with TLR agonist such as CpG oligoDNA or poly-IC RNA as an adjuvant. Frequency of vaccine-induced CTLs specific to each epitope was then determined by intracellular IFN-γ staining assay. Using similar mouse model, the effect of CHP nanogel delivery system on transportation of LPA to APCs in the draining lymph node (DLN) as well as T cell response to vaccination was also examined, comparing with a conventional delivery system, incomplete freund adjuvant (IFA).

Results: The IES candidates were selected based on in silico prediction of the sensitivity to proteasomal cleavage. The sensitivity of oligotyrosine and oligothreonine was predicted high, while that of oligoglycine and oligoproline was low. In the vaccinated mice, the LPA including the oligotyrosine IES most robustly stimulated specific CTLs towards all three epitopes included in LPA. The LPA including the oligothreonine IES was second best. In contrast, LPA including the oligoglycine or oligoproline IES often induced very limited CTL response. In the evaluation of delivery system, the CHP nanogel transported LPA to APCs in the DLN much more efficiently than IFA did. The kinetics of the CHP nanogel-mediated LPA transportation was closely similar to that of APC activation by TLR agonists, suggesting that the CHP nanogel harmonises the action of LPA and adjuvant. Indeed, in the presence of TLR agonists, the CHP nanogel greatly augmented the immunogenicity and anti-tumour effect of LPA vaccine.

Conclusions: We succeeded in the enhancement of immunogenicity of LPA vaccine by the rational design of IES in LPA and the CHP nanogel-mediated efficient antigen transportation to the DLN. These technologies will bring a remarkable improvement of vaccine efficacy.

P31

P57. Activation of RIG-I induces immunogenic cell death

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P31

Background: The interaction between the immune system and cancer cells has become a focus of recent cancer therapy research. Immunogenic cell death, short ICD, was described as a cell death modality that stimulates an immune response against dead-cell antigens, in particular when they derive

from cancer cells. Most malignant cells are poorly immunogenic and fail to elicit an effective antitumour immune response. Certain anti-cancer treatments, however, have been shown to induce ICD and transform cancer cells into potent inducers of an anti-cancer immune response. An important component is the induction of a specific cytotoxic T-cell response driven by DCs that have engulfed and processed tumour antigens. Recently, we have shown that the RIG-I ligand 3pRNA induces tumosr cell death *in vitro* and *in vivo*. Whether and how 3pRNA induced tumour cell death leads to a specific antitumor response is unknown. Here we analyse the immunogenicity of RIG-I induced tumour cell death *in vitro* and *in vivo*.

Material and methods: We induced tumour cell death by treating ovalbumin expressing B16 melanoma cells with 3pRNA. We co-cultured 3pRNA treated B16-OVA cells with splenic DCs and CFSE-labeled OT-I T-cells to analyse a specific T-cell activation and proliferation. Furthermore, we vaccinated 3pRNA treated B16-OVA cells subcutaneously into C57BL/6 mice to analyse their immunogenic potential *in vivo*. After vaccination, draining lymph node cells are analysed for T-cell activation and IFNg production using flow cytometry.

Results: We show that 3pRNA treatment leads to increased cytokine expression, upregulation of costimulatory molecules, cross-presentation and induction of cell death in B16-OVA melanoma cells *in vitro*. 3p-RNA treated B16-OVA cells induce proliferation and IFNg production of OT-I T-cells in co-cultures with spleen derived DCs. After subcutaneous injection of 3pRNA killed B16-OVA cells but not live B16-OVA cells into C57BL/6 mice, potent proliferation and IFNg production of antigen specific CD4 and CD8 T cells is seen in the draining lymph node. Overall, these effects were more pronounced after 3pRNA treatment than after Oxaliplatin induced cell death.

Conclusions: 3pRNA treatment of tumor cells leads to a potent immunogenic phenotype with induction of antigen-specific T-cell responses both *in vitro* and *in vivo*. These findings may have implications for a new therapeutic approach in immune mediated cancer treatment.

IMMUNOCYTOKINES

P32

P58. Can T-cells predict response to intravesical BCG immunotherapy in high-risk non-invasive bladder cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P32

Introduction: Intravesical BCG is an example of the importance of immunotherapy in cancer treatment. It has been used since the 1970s as it has a major impact in preventing or delaying bladder cancer recurrence and possibly progression. Unfortunately 20-30% of patients who receive this treatment do not respond and they are at high risk of dying from their disease. Being able to predict response to treatment would be an invaluable tool in those patients and would help in directing them to the appropriate treatment modalities. We investigated whether immunological markers in blood can predict outcome.

Methods: Patients with high risk non-invasive bladder cancer and due to have BCG immunotherapy were included. Blood samples were obtained before and after BCG-induction treatment. In vitro stimulation of peripheral-blood mononuclear cells with PPD which were then labelled with extra and intracellular markers in order to assess the differentiation and activation status of T-cells.

Results: 13 patients had no recurrence on follow-up cystoscopy while 6 had persistent disease. Differences were seen between the two groups in mean percentage of *Interferon gamma positive* (INFγ+) CD4 in response to PPD stimulation; 1.84% (±1.58) in the recurrence-free group versus 0.54% (±0.72) in the recurrence group [*P* value 0.0252]. This was more clear in the specialised subset [CCR7-CD27-CD28+]; 2.46% (±1.96) and 0.77% (±0.80) respectively [*P* value 0.0168]. And in the [CCR7-CD27-CD28+] subset; 5.07% (±4.19) and 1.12% (±1.25) respectively [*P* Value 0.0067]. The mean percentages of pre-treatment *tumour necrosis factor positive* (TNF+) cells were also significantly different in the [CCR7-CD27-CD28+] subset; 8.82% (±6.06) and 3.10% (±3.81) respectively [*P* value 0.0246].

Conclusion: In bladder cancer immunotherapy, the percentage of PPD inducible INFγ+ and TNF+ CD4 cells can potentially predict outcome to treatment.

COMBINATION THERAPY

P33

P59. Depleting the suppressors for the benefit of immunotherapy against cervical cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P33

Background: Cancer vaccines aim at inducing tumour-specific immune responses. However, in clinical studies so far these approaches have limited antitumour effect. Evidence is accumulating that MDSC (myeloid-derived suppressor cells) can suppress the antitumour immune response. Reversal of MDSC-mediated immune suppression by treatment with the tyrosine kinase inhibitor sunitinb can therefore possibly increase the efficacy of cancer vaccines.

Material and methods: We developed a method to assess MDSC depletion in a preclinical model of HPV-induced neoplasia. TC-1 (cells expressing HPV16-E7) tumour-bearing mice were injected with different dosages of sunitinib daily, for 9 days, with and without immunisation with SFVeE6,7 (Semliki Forest virus encoding human papilloma virus E6,7 tumor antigens). Intra-tumoral, intra-splenic and circulating MDSC and CD8 T cell levels were assessed after treatment.

Results: Upon sunitinib treatment, the absolute numbers of intra-tumoral, intra-splenic and circulating MDSC decreased in a dose-dependent manner. Combined sunitinib and immunisation therapy led to a marked decrease of intra-tumoral, intra-splenic and circulating MDSC levels as compared to non-treated control or immunisation alone. The bi-therapy regimen markedly enhanced intra-tumoral, intra-splenic and circulating levels of CD8 T cells. The highest number of circulating CD8 T cells undergoing degranulation (CD107ab+) was observed after combined treatment. Most importantly, this combined sunitinib and immunisation treatment regimen abrogated tumour growth.

Conclusions: In summary, we demonstrated that sunitinib alone or in combination with immunisation can decrease intra-tumoral, intra-splenic and circulating MDSC levels. Also, combination of sunitinib treatment with immunisation enhanced levels and degranulating activity of CD8 T cells, thus resulting in reversal of tumour growth. This study indicates that SFV-based immunotherapy combined with sunitinib treatment could improve treatment outcome.

P34

P60. Microtubule-depolymerising agents used in antibody-drug-conjugates induce anti-tumour immunity by stimulation of dendritic cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P34

Antibody drug conjugates (ADCs) are emerging as powerful treatment strategies with outstanding target specificity and high therapeutic activity in cancer patients. Brentuximab vedotin represents a first-in-class ADC directed against CD30-positive malignancies. We hypothesised that its sustained clinical responses could be related to the stimulation of an anti-cancer immune response. We here demonstrate that the dolastatin family of microtubule inhibitors, from which the cytotoxic component of brentuximab vedotin is derived, comprises potent inducers of phenotypic and functional DC maturation. In addition to the direct cytotoxic effect on tumour cells, dolastatins efficiently promoted antigen uptake and migration of tumour-resident DCs to tumour-draining lymph nodes. Exposure of murine and human DCs to dolastatins significantly increased their capacity to prime T cells. Underlining the requirement of an intact host immune system for

the full therapeutic benefit of dolastatins, the anti-tumour effect was far less pronounced in immune-compromised mice. When combining dolastatins with tumour-antigen-specific vaccination or blockade of the PD-1/PD-L1 and CTLA-4 co-inhibitory pathways, we observed substantial therapeutic synergies. Ultimately, ADCs using dolastatins induce DC homing and activate cellular anti-tumour immune responses in patients. Our data reveal a novel mechanism of action for dolastatins and provide a strong rationale for clinical treatment regimens combining dolastatin-based therapies, such as brentuximab vedotin, with immune-based therapies.

P36

P62. Effectiveness of melatonin, IL-25 and siRNA IL-17B in growth control of breast cancer cell lines

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P36

Interleukin (IL)-25, a cytokine active in inflammatory processes and production of proinflammatory cytokines, is secreted by mammary epithelial non-malignant cells and induces apoptosis in tumour cells by differential expression of its receptor IL-17RB, highly expressed in malignant cells and lower expressed in non-malignant epithelial cells. It is known that another ligand (IL-17B) compete for the same site of action in tumour cells, contributing to its tumorigenic potential. The melatonin hormone acts in several activities, among them the immune system regulation, proliferation, differentiation and apoptosis process in tumour cells. The aim of this study was to evaluate the effects of treatment with IL-25, silencing gene (siRNA) of cytokine IL-17B and melatonin in human mammary tumour cells for the control of cell proliferation and induction of apoptosis. Non-metastatic mammary tumour cells (MCF-7), metastatic (MDA-MB-231) and a human normal mammary epithelial cells (MCF-10A) were cultured and divided into five treatment groups: group I control, group II treated with IL-25 protein, group III treated with siRNA IL-17B, group IV treated with melatonin and group V joint treatments. The gene silencing was standardised by real-time PCR (qPCR), cell viability assessed by MTT assay and protein expression of caspase-3, apoptotic marker, by immunocytochemistry and subsequent quantification by optical densitometry. After 24 hours of incubation with IL-25 at concentrations of 1, 10 and 50 ng/mL was found a significant decrease in cell viability at 1 ng/mL (38.5 % for MCF-7 cells and 74.0 % for MDA-MB-231 cells; $p<0.05$) compared with control group, and increased expression of caspase-3 ($p<0.001$) in both lineage cells. The treatment with siRNA IL-17B at 10nM significantly decreased cell viability (80.0% for MCF-7 cells and 86.0% for MDA-MB-231 cells, $p<0.05$), and showed a slight increase in the expression of caspase-3. When treated with melatonin at concentrations from 0.001 to 1 mM was observed a significant decreased in cell viability at 1 mM (70.0% for MCF-7 cells and 41.0% for MDA-MB-231 cells; $p<0.05$) and high expression of caspase-3 ($p<0.001$) in both lineage cells. For MCF-10A cells, there was no decrease in cell viability to treatments proposed, enabling the use of these therapeutic agents. The joint treatments showed synergic action in reducing the cell viability and induction apoptosis. Our results reinforce the anti-proliferative properties of these agents in breast cancer, establishing new therapeutic strategies. In addition, understanding the role of these factors in immune modulating highlights the growing connection between the contribution of the immune system to combat cancer progression.
Financial support: FAPESP

ADOPTIVE IMMUNOTHERAPY

P37

P63. Autologous tumour cells and SW742 allogeneic cell line have comparable stimulating effect on PBMCs of gastrointestinal malignant patients in vitro

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P37

Background: Natural killer activity is believed to be important contributor of a patient's immune system to fight cancer. However, cancer patients have reportedly defective NK activity and the malignant target frequently has developed mechanisms to escape detection of NK cells. Our research is aimed at overcoming this NK cell deficiency.
Materials and methods: Malignant autologous epithelial cells of 10 colorectal carcinoma patients were separated by cell culture procedures. Peripheral blood mononuclear cells (PBMCs) were stimulated with their mitomycin treated autologous tumour cells or allogeneic SW742 colorectal carcinoma cell line. The expression of CD3, CD56, NKG2D and NKp44 were detected with flowcytometry and reverse transcription-PCR. NK activity of PBMCs against K562 target cell line was measured by MTT colorimetric assay.
Results: Stimulation with autologous tumour cells and allogeneic SW742 colorectal carcinoma cell line augmented CD56+ and CD56+CD3+ cells and up-regulated NKG2D and NKp44 expression. NK activity of PBMCs after co-incubation with autologous tumour cells or SW742 was significantly raised.
Conclusions: Our results demonstrated that stimulation of PBMCs by SW742 can significantly improve NK activity as much as by autologous tumour cells which was confirmed by the higher expression of NKp44 and NKG2D. Since the separation of autologous tumor cells is difficult and time consuming the allogeneic tumour cell line could be a good replacement for large scale short term generation of activated NK cells. These data may help to improve cancer immunotherapy protocols.

P38

P64. T cell re-direction against Glypican-3 for immunotherapy of hepatocellular carcinoma

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P38

Hepatocellular carcinoma (HCC) is the third most common cause of cancer related mortality world-wide and therapeutic options are very limited. A new therapeutic approach is the adoptive T cell therapy of HCC. Glypican-3 (GPC3) as a tumour associated antigen is expressed in up to 60% of all HCC but not in healthy human liver tissue. Therefore, our goal is to generate cytotoxic T lymphocytes (CTL), which are capable of recognizing and eliminating GPC3-expressing tumor cells.
Immunodominant epitopes for GPC3 have not been described yet. In this study we used Ultra Nano HPLC coupled on-line to the Q Exactive mass spectrometer to obtain a comprehensive HLA class I peptidome from a GPC3 and HLA-A2 positive hepatoma cell line. The resulting data were analysed using the MaxQuant bioinformatics platform. Two HLA-A2 bound GPC3 peptides could be identified, later on referred to as GPC3-P1 and GPC3-P2. These results enable us to target GPC3 epitopes that are presented on GPC3 positive HCC cells.
To isolate tumour reactive high avidity T cells, an allo-restricted stimulation approach was used. For stimulation of naïve T cells, autologous dendritic cells were co-transfected with GPC3 and HLA-A2 RNA and used as antigen presenting cells. T cells from the naïve T cell repertoire of HLA-A2 negative donors were co-cultured with and expanded on these HLA-A2+ GPC3+ DCs. After two weeks, MHC streptamer-positive $CD8^+$ T cells specific for both targeted GPC3 epitopes were detected (<1%). We were able to enrich these cell populations further to 35% GPC3-P1- and 57% GPC3-P2-MHC streptamer-positive T cell lines and grew T cell clones from them. In a co-culture with GPC3-P1/ -P2 peptide loaded T2 cells we identified T cell clones displaying specific effector function by IFNγ secretion. Functional T cell clones showed strong GPC3 MHC streptamer binding.
We have cloned the first T cell receptors (TCR) to either GPC3 peptide from these T cell clones. T cells engrafted with the GPC3 specific TCRs showed strong GPC3 MHC streptamer binding. When co-cultured with

GPC3 peptide loaded target cells or a GPC3 expressing hepatoma cell line (HepG2), GPC3 TCR transduced T cells secreted IFNγ. Furthermore cytotoxicity was observed by killing of up to 60% of HepG2 cells. GPC3-directed T cell therapy shows great promise for the treatment of HCC.

P39

P65. Minor-histocompatibility-antigen UTY as target for graft-versus-leukaemia and graft-versus-haematopoiesis in the canine-model

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P39

Background: In haploidentical-SCT male-patients with female-donors have better prognosis compared to female-to-male-combinations due to Y-encoded minor-histocompatibility-antigens recognised by female-allo-immune effector-lymphocytes in the context of a graft-versus-leukaemia-(GvL)-effect. We provide data in a dog-model that the minor-histocompatibility-antigen UTY might be a promising target to further improve GvL-immune-reactions after allogeneic-SCT.
Materials and methods: Canine (c) purebred-beagle-dogs' PB and BM were studied. T2-cells (HLA-A2+, TAP-deficient) were used. These human-(h)-UTY-sequence-derived HLA-A2-binding-peptides were investigated: W248 (WMHHNMDLV), T368 (TLAARIKFL), K1234 (KLFEMIKYC). ***In vitro:*** Autologous-cDCs were generated with best of three DC-methods (*Calcium-Ionophore*, *Picibanil*, *Cytokines*). Generation cUTY-specific-CTLs: CD3+ T-cells were co-cultured with autologous-mature cDCs+hUTY-peptides (weekly restimulation for 21 days; +hIL-2, +hIL-7). Cytotoxicity and antigen-specificity were determined by [^{51}Cr]-release- and cIFN-g-ELISPOT-assays. Cells were quantified day 0 and of harvest using anti-cmAbs/hmAbs (FACS), UTY-mRNA-expression via RT-PCR-analysis. ***In vivo*:** A female-dog was immunised with PBMCs from a DLA-identical-male-dog (day 0 and 14). PB-derived T-cells were harvested 35 days post 2nd-injection followed by analysing UTY-specific-reactivity.
Results: Female cUTY-specific-CTLs were stimulated *in vitro* using autologous-DCs loaded with three HLA-A2-restricted UTY-derived-peptides (≤2.9-fold-expansion) and specific T-cell-responses were determined in 3/6 female-dogs. CTLs specifically recognised/lysed autologous-female peptide-loaded-DCs (900 spots/100,000 T-cells (median)/≤47.9%), but not naive autologous-female-DCs and -monocytes ($p \leq 0.026$). They mainly recognized BM and to a lower extent DCs, monocytes, PBMCs and B-cells from DLA-identical-male-littermates and peptide-loaded T2-cells in an MHC-I-restricted manner (up to $p \leq 0.046$). UTY-mRNA was only expressed in male-cells. A UTY-/male-specific-reactivity was also obtained *in vivo* after stimulation of a female-dog with DLA-identical-male-PBMCs.
Conclusions: We demonstrated natural UTY-processing/presentation in dogs. Female-dog-CTLs were specifically stimulated by HLA-A2-restricted-UTY-peptides, thereby enabling recognition of DLA-identical-male-cells, mainly BM-cells. These observations suggest UTY as a promising candidate-antigen to improve GvL-reactions in the course of immunotherapy. Next-generation-sequencing and specialised-bioinformatics-algorithms are now focus for human-individualised-leukaemia-treatment (T-cell-receptor-Profiling, detection/selection of T-cell-receptor-clones or DC-based-immunotherapies).

P40

P66. Generating and characterising WT1-specific T cells – research towards adoptive tumour therapy

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P40

Background: The Wilms tumour antigen 1 (WT1) is a self-antigen expressed at high levels in leukaemic cells, but not in healthy tissue. WT1, therefore, is a favourable target antigen for allogeneic T cell therapy to prevent leukaemic relapse after stem cell transplantation. However, a comprehensive characterisation of CD4$^+$ and CD8$^+$ WT1-specific T cells is missing and the efficient expansion of a polyclonal WT1-reactive T cell population for clinical use has remained a major challenge.
In this study we aim to directly *ex vivo* characterize WT1-specific T cells present in the blood of healthy donors at high-resolution and to develop a method for the rapid generation of functionally potent, polyclonal CD4$^+$ and CD8$^+$ WT1-specific T cells for clinical use.
Methods: We utilise the magnetic enrichment of activation marker expressing cells after antigen-specific stimulation, as low frequencies of WT1-specific T cells in healthy donors do not allow direct detection.
Results: *Ex vivo* frequencies of WT1-specific T cells range between 10^{-6} and 10^{-5} WT1-specific T cells within CD4$^+$ T cells. In about 80% of healthy donors (n=15) a CD4$^+$ memory response, accompanied by production of effector cytokines like IFN-γ and TNF-α against WT1 peptides is present. In contrast, detected CD137$^+$CD8$^+$ WT1-reactive T cells exhibit a naïve phenotype (CD45RA$^+$CCR7$^+$) in all tested donors (n=5).
An improved short-term expansion protocol to generate potent WT1-specific T cell cultures for clinical use was established utilising a CD137$^+$ cell enrichment step. Notably, a high frequency of expanded CD4$^+$ and CD8$^+$ T cells show specific reactivity against WT1-presenting autologous cells as detected by production of effector cytokines after antigen-specific restimulation. Cytotoxic activity against antigen-loaded target cells could be shown by direct flow-cytometry-based cytotoxicity assays and antigen-specific upregulation of the degranulation marker CD107a. WT1-MHCI-Tetramerstainings furthermore confirmed antigen-specificity and suggested polyclonality within the CD8$^+$ T cell population. In contrast to previous expansion protocols our polyclonally expanded T cells exhibit a favourable, unexhausted memory phenotype, express co-stimulatory markers CD27 and CD28 and the IL7R-a chain (CD127).
Conclusions: Functional, polyclonal CD4$^+$ and CD8$^+$ WT1- reactive T cells can be efficiently enriched directly *ex vivo* from the natural repertoire by magnetic separation of T cells after antigen-specific stimulation. Thus, our approach holds great potential for the GMP-compliant generation of WT1-specific T cells for future clinical use.

P41

P67. Targeted natural killer (NK) cell based adoptive immunotherapy for the treatment of patients with non-small cell lung cancer (NSCLC) after radiochemotherapy (RCT) – clinical application of NK cells activated by heat shock protein 70 (Hsp70)

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P41

Membrane-bound Hsp70 serves as a recognition structure for NK cells that were pre-stimulated with Hsp70 peptide TKD plus low dose IL-2 *in vitro* and in mouse models. In a clinical phase I trial feasibility, safety and tolerability of *ex vivo* TKD/IL-2 stimulated autologous NK cells has been demonstrated in patients with metastasised colorectal carcinoma and NSCLC. Based on these findings a proof-of-concept phase II randomised clinical trial was initiated (BMBF - Innovative therapies). NSCLC patients will be treated with ex vivo stimulated NK cells after RCT. Most patients are diagnosed in locally advanced disease stages IIIA and IIIIB. After conventional radiochemotherapy only part of the patients (less than 50%) show remission and despite improvements in standard therapies the mortality associated with this disease is very high (5 year survival rate does not exceed 15%). Therefore there is a strong medical need for innovative treatment strategies. Since an Hsp70 membrane-positive tumour phenotype is associated with a poor clinical outcome, only Hsp70 membrane-positive tumour patients are recruited into the trial. Leukapharesis products are generated centralised and cell processing is performed in a GMP-laboratory.
The aim of the study is to show the efficacy of the treatment with Hsp70-peptide TKD/IL-2 activated, autologous NK cells following completion of standard RCT by improvement of PFS.

ENGINEERED T-CELL THERAPY

P42

P68. A new EGFR - EpCAM bispecific antibody enhances the efficacy of adoptive T-cell therapy in a murine gastric tumour model

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P42

Background: A limiting step for adoptively transferred tumour-specific T cells is their recruitment from the blood circulation to the proximity of tumour cells and subsequent engagement in direct tumour cell contact. We hypothesised that a bispecific antibody recruiting T cells to a target antigen on tumour cells could enhance T-cell-tumour interaction and thus increase the efficacy of adoptively transferred T cells.
Material and methods: A new bispecific murine IgG2a antibody (BsAb) was generated that recognises EpCAM as a tumour antigen and truncated EGFR (D-EGFR) as an inert surface marker protein on transduced T cells. T cells from transgenic mice for TCR specific for the SV40 large T antigen (TCR-1) were retrovirally transduced with D-EGFR. S.c. tumors were induced in C57Bl/6 mice by injecting mGC8 cells derived from a syngeneic large T antigen expressing EpCAM-positive gastric tumor.
Results: *In vitro*, the BsAb increased (4-fold) binding of transduced T cells to EpCAM positive tumour cells. In the presence of the BsAb, tumour-directed T cells efficiently lysed EpCAM-positive cells (83 % at a 10:1 effector to target ratio). *In vivo*, the antibody reached EpCAM+ tumour cells as evidenced by immunofluorescence. mGC8 tumour-bearing mice were treated twice with a combination of the BsAb and transduced TCR-I T cells. Tumour growth was significantly reduced for over 30 days (n=12) compared with control groups (transduced T-cells or BsAb alone) and survival was prolonged by > 30 days ($p<0.001$).
Conclusions: Co-administration of a BsAb bridging adoptively transferred tumour-specific T cells via an inert surface molecule to a tumour-associated surface antigen enhances the efficacy of therapeutic T cell transfer.

P43

P69. Targeting naturally presented, leukemia-derived HLA ligands with TCR-transgenic T cells for the treatment of therapy refractory leukemias

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P43

Background: T cells have proven to be effective for the treatment of leukaemias in form of donor lymphocyte infusions (DLI). However, those DLI are of unknown specificity and often associated with graft versus host disease. New effector tools in form of T cell receptor (TCR)-transgenic T cells with specificity for leukaemia-derived human leukocyte antigen (HLA) ligands are highly promising tools for the treatment of life-threatening, therapy refractory leukaemias.
Material and methods: In order to identify leukaemia-derived HLA ligands, we performed the immunopeptidomic approach on seven samples from patients with myeloproliferative neoplasias (MPN). HLA ligands derived from genes with expression restricted to the hematopoeitc system were identified by database searches and literature research and validated by quantitative PCR (qPCR) and immunohistochemistry (IHC). For isolation of peptide-specific TCR, naive T cells of healthy blood donors were primed with single HLA-mismatched dendritic cells, pulsed with synthetic counterparts of selected HLA ligands. Peptide specific T cells were isolated using HLA multimers and cloned by limiting dilution. TCR were isolated out of peptide-specific T cell clones and characterised for their *in vitro* and *in vivo* leukaemia reactivity as well as their *in vitro* on- and off-target reactivity.
Results: Using the immunopeptidomic approach on seven MPN samples, we were able to identify 4386 unique HLA ligands. Nineteen of those ligands are derived from seven genes with restricted expression to the hematopoietic system and are presented on six different HLA molecules. We exemplarily selected the antigen myeloperoxidase (MPO) (five identified ligands) and confirmed restricted expression to myeloid cells. A TCR (named TCR2.5D6) with high specificity for the HLA-*B*07:02* restricted ligand MPO_5 could be isolated. TCR2.5D6-transgenic T cells show *in vitro* leukaemia reactivity including colony forming leukaemic progenitor cells. Strikingly, anti-leukaemic reactivity could be observed in a murine model of human acute myeloid leukaemia with HLA-*B*07:02*-transgenic NB4 cells, resulting in significantly prolonged survival and reduced bone marrow infiltration by leukaemic cells. However, strong immune pressure led to the development of tumours that lost transgene expression in the TCR treated group. Extensive experiments regarding safety of the TCR2.5D6 revealed lack of reactivity against MPO^- cells including healthy hematopoietic stem cells. Experiments with alanine variants of the MPO_5-peptide resulted in a recognition pattern that is unique for the MPO-specific peptide.
Conclusion: In conclusion, as shown for the MPO-specific TCR, combination of the immunopeptidomic identification of leukaemia-derived HLA ligands with the isolation of TCR in the single HLA-mismatched setting is suitable to generate highly specific, leukaemia-reactive TCR-transgenic T cells. Further TCR are isolated and characterized at the moment to allow therapeutic application for a broad patient population.

P44

P70. Development of clinically implementable imaging strategies for tracking T cell receptor-transgenic T cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P44

Transfer of T lymphocytes genetically modified with T-cell receptors (TCR) specific for tumour-associated antigens is a novel therapeutic approach for diverse malignant diseases. However, efficacy as well as safety of this therapy still needs to be improved. In order to understand T cell trafficking and functionality in vivo, the development of non-invasive and sensitive cell-tracking technologies would be of high value. Moreover, clinical translation of such technologies would further provide possibilities to improve this therapeutic approach in humans. We aim to establish a non-invasive, clinically translatable imaging approach for in vivo monitoring of adoptively transferred T cells engineered with selected TCRs. We have previously isolated several TCRs specifically recognising peptides derived from diverse tumor associated antigens and selected one TCR recognising lymphoma cells with defined HLA-DR restriction.
Peripheral blood mononuclear cells from healthy donors were retrovirally transduced with the selected TCR. To track transduced T cells, we used an anti-murine TCRβ monoclonal antibody (TCRmu), which binds to the murinzed region of the introduced TCR. This antibody was either radioiodinated with Iodine-124 or conjugated with a bifunctional chelator and labelled with Zirconium-89. Labelled antibodies were tested for stability and specific binding in vitro. A xenogenic mouse model was established using Nod/SCID mice injected intraperitoneally with lymphoma cells. After tumour inoculation, we transferred TCR-transduced human T cells and PET imaging was performed at different time points post injection of ^{124}I-TCRmu or ^{89}Zr-TCRmu. Specific in vivo binding was evaluated by co-injection of an excess of unlabelled antibody or isotype control antibody. In vivo uptake was confirmed by autoradiography and immunostaining for human CD3 on tumour frozen sections.
We established labelling of TCR transduced T cells using a specific antibody (TCRmu) marked with Iodine-124 or Zirconium-89. After the radio-labelling, affinity and specificity of the antibody was maintained while viability and functionality of T-cells remained unaffected. In vivo imaging of TCR-transduced T cells in the xenograft tumor model revealed

strong uptake on the tumour area. Improved signal detection and reduced background was observed using ^{89}Zr-anti-TCRmu. These results correspond to autoradiographic signals and detection of human T cells on the tumour border. Injection of an excess of unlabelled TCRmu showed depletion of human T cells in vivo, enabling a possible approach to control potentially autoreactive T cells in vivo.
In summary, we developed a non-invasive imaging model for tracking specifically human TCR-engineered lymphocytes in vivo. This model will be useful to monitor adoptive transfer of TCR transgenic T cells in vivo and therefore giving important information for further optimisations regarding efficacy and safety of immunotherapeutic approaches.

P45

P71. Adoptive transfer of TCR gene-transduced lymphocytes targeting MAGE-A4 for refractory esophageal cancer

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P45

Background: Engineering the antigen receptor gene in patients' lymphocytes is one promising strategy to create antigen-specific lymphocytes without senescent phenotypes. The strategy provides an opportunity to extend the application of adoptive T cell therapy for cancer patients. However, this concept has not been tested in the epithelial cancer patients.
Materials and methods: MAGE-A4-specific TCR α and β chains were cloned from a human T cell clone that recognises MAGE-A4$_{143\text{-}151}$ peptide in a HLA-A*24:02 restricted manner. This T cell clone did not show any cross reactivity to the peptides with homology to the MAGE-A4$_{143\text{-}151}$ epitope. A retroviral vector that encodes these TCR chains without any artificial modification was constructed; the lymphocytes transduced with the retroviral vector killed the MAGE-A4 expressing tumor in vitro and inhibited the tumour growth in the NOG immunodeficient mice.
A phase I clinical trial of TCR gene therapy targeting MAGE-A4 was performed to treat refractory esophageal cancer patients without lympho-depleting pre-conditioning. The trial was designed as a cell-dose escalation consisting of three cohorts, 2x10^8, 1x10^9 and 5x10^9 cells/patient. Vaccines with the cognate peptide were also given following adoptive transfer of lymphocytes on day 14 and day 28.
Results: The treatment was tolerable with no adverse events associated with transferred cells or any viral toxicity. In all ten patients of the 3 cell-doses, the transferred lymphocytes were detected in their peripheral blood in a dose-dependent manner during the first 14 days. In 4 patients, the infused cells have been persisting more than 5 months after the transfer. The transferred lymphocytes that were harvested from the patients more than 50 days after the transfer were found to sustain the reactivity to the antigen-expressing tumour cells. Three patients showed SD or long tumour free status.
Conclusions: This approach may extend the availability of adoptive T cell therapy for epithelial cancer patients by providing tumour-reactive and long surviving lymphocytes.

P46

P72. Transgenic expression of a chimeric signaling receptor to facilitate T cell costimulation in the tumour environment

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P46

Tumour therapy with T cell receptor (TCR) engineered T cells is reported to induce clinical responses but shortcomings regarding poor *in vivo* persistence and loss of function in the tumour milieu have been observed. Providing costimulation to adoptively transferred T cells may improve these shortcomings. However, human T effector cells are largely CD28 negative and epithelial tumours do not express CD80 or CD86. Therefore, costimulation of human CD8 T effector cells cannot be triggered via the classical way of CD28 ligation. We propose to facilitate costimulation of CD8 T effector cells in the tumour milieu through retroviral engineering of T cells with a chimeric signaling molecule (CSM). This CSM is consisted of an intracellular costimulatory domain fused to an extracellular domain with binding capacity for a ligand expressed by a great variety of tumours.
Human activated PBL retrovirally transduced to express the CSM exhibited a survival advantage during *in vitro* expansion according to clinical protocol. The effect of the chimeric molecule on T cell function was analyzed using T cells expressing a tumour antigen specific TCR alone or in combination with the CSM. Transduced T cells were stimulated with target cells positive or negative for the CSM ligand (CSM-L). CSM expressing T cells responded better to CSM-L$^+$ target cells showing higher phosphorylation of ERK and RPS6 compared to stimulation with CSM-L$^-$ target cells. CSM$^-$ T cells responded equally to both target cells. Accordingly, CSM$^+$ but not CSM$^-$ T cells secreted more IL-2 and IFN-γ upon co-culture with CSM-L$^+$ target cells. In summary, transduction of PBL with the chimeric signaling molecule supported T cell survival and TCR induced signaling leading to enhanced T cell function.

P47

P73. Functional characterisation of HBV-specific T cell receptors for redirection of T cells against HBV infected hepatocytes

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P47

Chronic HBV infection, which is accompanied by a weak and oligoclonal T cell response, is the most common cause of hepatocellular carcinoma (HCC). Current antiviral therapies do not eliminate the virus, but T cell therapy will very likely do so. From PBMCs of two HLA-A2$^+$ acutely infected patients and a donor who cleared HBV infection we have established several HBV-specific monoclonal T cell lines. Thereof we isolated 11 different T cell receptors (TCR) that are specific for the HBV S-protein derived peptides S20 (FLLTRILTI) and S172 (WLSLLVPFV) or for the C18 core-peptide (FLPSDFFPSV). The aim of this study was a functional comparison of our set of HBV-specific TCRs in order to identify TCRs with optimal recognition of HBV peptides presented on HLA-A2.
By murinization and codon-optimisation of gene sequences of TCR a and b chains, fused by a P2A element for polycystronic expression, TCR expression after retroviral transduction was increased 2-fold to 60% of PBMCs expressing an HBV-specific TCR.
PBMCs transduced with the 11 optimised HBV-specific TCRs were compared in killing assays using peptide-pulsed T2 cells, LCLs and HBV-replicating HepG2.2.15 cells as targets. CD8$^+$ T cells transduced with the core-specific TCRs killed target cells loaded with 0.01 nM of peptide. Cells specific for the S20 and S172 peptide were less sensitive with a specific lysis as low as 0.1 nM. Expression of most of the HLA-A2 restricted HBV-specific TCRs in CD4$^+$ T cells also led to specific cytotoxicity, which was 10-fold reduced in sensitivity compared to CD8$^+$ T cells and independent of CD8 co-receptor binding. Notably, our HBV-specific TCRs recognised peptide presented on various different HLA-A2 subtypes.
CD8$^+$ T cells transduced with HBV-specific TCRs were also able to recognise endogenously processed peptides and specifically kill HBV-replicating hepatoma cells and strongly reduce cccDNA levels in HBV-infected HepaRG cells.
In addition, intracellular cytokine staining after stimulation showed that the TCR-transduced CD8$^+$ T cells were polyfunctional, secreting INF-γ, TNF-α and IL-2, whereas CD4$^+$ T cells produced mainly TNF-a and/or IL-2.
We will further analyse our HBV-specific TCRs in HBV/HLA-A2 transgenic mice in order to identify the TCR that confers best antiviral activity. Our HBV-specific TCRs may be used for elucidating specific anti-HBV mechanisms exerted by T cells, and most importantly, for adoptive T cell therapy of chronic hepatitis B and HBV-induced HCC.

P48

P74. A Good Manufacturing Practice procedure to generate therapeutic numbers of highly pure anti-leukaemic virus-specific T-cells

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P48

Background: Recently, we have started a clinical trial to treat patients with high risk acute leukaemia with a donor-derived HA-1-TCR transduced virus-specific T-cell product as early as 8 weeks and 14 weeks after allogeneic stem cell transplantation (allo-SCT). Donor derived Cytomegalovirus (CMV)- and Epstein Bar virus (EBV)-specific T-cells will be isolated using Streptamer based CliniMACS selection, and will be subsequently transduced at day 2 with the well-characterized anti-leukaemic HA-1-TCR and infused 10-12 days later. Based on these well-defined specificities this T-cell product is predicted to result in a selective Graft versus Leukaemia (GvL) effect without Graft versus Host Disease (GvHD). Important study parameters are persistence of the T-cell product, feasibility of generation of HA-1-TCR transduced virus-specific T-cells, and the number of events of acute GvHD.

Material and methods: To obtain therapeutic cell numbers, one of the inclusion criteria is presence in donor peripheral blood of 1 or 2 virus-specific T-cell population with a frequency of ≥0.05% of T-cells. MHC-Streptamers will be used to isolate 1 or 2 virus-specific T-cell populations from donor leukocytes. MHC-Streptamer incubation will result in binding of the TCR of the virus-specific T-cells of interest to the specific peptide presented by the MHC molecule on the Streptamers. Next to allowing selection of T-cells of interest, this binding will also result in specific stimulation allowing subsequent transduction with the HA-1-TCR. The process of isolation of pure populations of virus-specific T-cells and transduction with good manufacturing practice (GMP)-grade retroviral supernatant encoding the HA-1-TCR has been validated with 4 large scale test procedures in the cleanroom. To pass the in process (IP) testing, T-cells needed to be ≥50% pure for the respective virus-specific tetramer directly after Streptamer isolation. In addition, after transduction and subsequent culturing T-cells need to be ≥60% antigen-specific, as measured with virus- and HA-1-tetramers. Moreover, transduction efficiency of ≥5% as measured with HA-1-tetramers is a prerequisite.

Results: All HA-1-TCR td virus-specific T-cell products met the criteria for IP testing and quality control testing. They contained >90% antigen-specific T-cells and >10% HA-1 tetramer positive T-cells. Moreover, all HA-1-TCR td virus-specific T-cell products were highly reactive against HA-1-positive leukaemic cells.

Conclusions: Here, we present a GMP-grade procedure to generate in a short culture period of less than 2 weeks therapeutically relevant numbers of defined antigen-specific and highly anti-leukaemia reactive T-cells.

P49

P75. Genetic engineering of T cells for increased homing to the tumor site

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Journal for ImmunoTherapy of Cancer 2014, **2(Suppl 2):**P49

Adoptive cell transfer (ACT) using in vitro expanded T cells from biopsy material represents a highly promising treatment of disseminated cancer. ACT in its present form is rather crude and improvements seem within reach. Recruitment of transferred lymphocytes to the tumor site is a crucial step in ACT efficacy; however, quite few T cells actually reach the tumor site upon administration. In the present pre-clinical study we have genetically engineered T cells aiming at increasing the homing of T cells by matching expression of chemokine receptors on T cells to chemokines secreted by the tumor, thus improving anti-tumor efficacy of ACT. By PCR analysis we found that several malignant melanoma (MM) cell lines showed expression of cytokines CXCL8/IL-8, CXCL12/SDF-1 and CCL2, which was confirmed by ELISA analysis of MM conditioned medium. Taking advantage of mRNA electroporation we successfully transfected T cells with mRNA encoding the chemokine receptors CXCR2 and chimeric receptor CXCR4-R2 on the cell surface, the latter expressing the intracellular region of CXCR2 allowing expression in T cells. Work is in progress, but so far chemokine receptor CXCR2 and chimeric receptor CXCR4-R2 transfected T cells are capable of migrating towards their ligands, CXCL8 and CXCL12 respectively, in *in vitro* transwell migration assays. *In vitro* studies on the transfection and function of the CXCR4 and CCR2-R2 chimeric receptors as well as *in vivo* migration studies have been initiated, and data will be presented at the meeting.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Idorn *et al.*: P75. Genetic engineering of T cells for increased homing to the tumor site. *Journal for ImmunoTherapy of Cancer* 2014, 2(Suppl 2):P49

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March 2014

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