895 Dasatinib-induced reduction of tumor growth is accompanied by the changes in the immune profile in melanoma B16.OVA mouse model

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Background: Dasatinib, a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukemia and acute lymphoblastic leukemia, has been shown to have immunomodulatory effects in addition to direct oncokinase inhibition. Recently, we observed that dasatinib induces a rapid and marked mobilization of lymphocytes, which closely follows the drug plasma concentration. In addition, in a subgroup of patients a clonal expansion of large granular lymphocytes occurs, which is correlated with good therapy response.
As dasatinib-induced immunomodulatory effects are not leukemia specific, we aimed to characterize the anti-tumor immune responses in a syngeneic murine melanoma model.
Materials and Methods: B16.OVA cells were implanted subcutaneously to C57BL/6J mice. The mice (n = 6/group) were treated daily i.g. either with 30 mg/kg dasatinib or vehicle only. Blood was collected before tumor transplantation, before treatment, and on treatment days 4, 7 and 11. Tumor volumes were measured manually and specific growth rate was calculated based on the first and the last day of the treatment. In addition to white blood cell differential counts, immunophenotyping of blood and tumor homogenate was done by flow cytometry α-CD45.1, -CD3, -CD4, -CD8b and -NK1.1, and -SIINFEKL-pentamer to detect B16.OVA-specific cells. To assess the functional properties of lymphocytes, we used antibodies against CTLA-4 and PD-1, and CD107. In addition, to study whether dasatinib has an effect on the cytotoxicity of the naïve or the TCR-specific cytotoxic cells we incubated splenocytes isolated from wild-type mouse and OT-I mouse spleen with B16.OVA cells in presence of 100 nM dasatinib.
Results: On the 11th day of treatment, the tumor volumes were smaller in dasatinib group compared to control group, and there was a significant decrease in the tumor growth rate (0.06 vs. 0.18, p = 0.01). Furthermore, dasatinib treated mice had increased proportion of CD8+cells in the circulation (17.9% vs. 14.4%, p = 0.005) and the CD4/CD8 ratio was significantly decreased (1.39 vs. 1.52, p = 0.04). During the tumor growth the mean CTLA-4 expression on CD8+ cells increased from 1.2% to 9% in the control group, whereas, in dasatinib group the increase was more modest (1.2% to 5.7%). Interestingly, 80% of tumor infiltrating CD8+ cells expressed PD1 antigen compared to <5% of PD1 positive CD8+ cells in the peripheral blood suggesting lymphocyte anergy or exhaustion induced by tumor cells.
The results from the cytotoxicity assay are in accordance with the *in vivo* findings and showed a significant enhancement in the cytotoxicity of both naïve and OT-1 T-cells specifically due to dasatinib.
Conclusions: To conclude, dasatinib treatment slowed down the tumor growth in B16.OVA mouse model, which could be associated with the immunomodulatory effects of dasatinib. However, combinatorial treatment regimens may be needed as dasatinib is not able to fully break the immune cell anergy induced by tumor cells.
Conflict of interest: *Other substantive relationships: Akseli Hemminki is shareholder in Oncos Therapeutics, Ltd. Akseli Hemminki is employee and shareholder in TILT Biotherapeutics Ltd. Kimmo Porkka and Satu Mustjoki have received research funding and honoraria from Novartis and Bristol-Myers Squibb, but these are not related to this study.*

896 Differentially expressed functions and genes between serrated adenocarcinoma and sporadic colorectal carcinoma showing histological and molecular features of high level of microsatellite instability

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Introduction: Serrated polyp pathway is considered as an alternative pathogenic route for non-conventional colorectal carcinomas (CRCs) accounting for around 30% of CRC. Serrated polyps are characterised by the high frequency of microsatellite instability (MSI) and BRAF mutation and are commonly found adjacent to serrated adenocarcinomas (SAC), a recently described histological subtype of CRC showing worse prognosis and less peritumoural lymphocytic infiltrate than conventional CRC. However, previous independent studies have demonstrated that SAC are generally BRAF-native and microsatellite stable (MSS). Both SAC and CRC with molecular and histological features of high-level of MSI (MSI-H) have been proposed as end-points of the serrated polyp pathway but to date, there are no molecular profiling studies assessing possible different functions and genes between these two histological CRC subtypes.
Materials and Methods: The study population was based on a previous series of CRCs. Twenty-two frozen SACs and 9 sMSI-H specimens were selected for this study. These cases were matched for age, gender, location, Dukes' stage and WHO grade. RNA extraction was performed using Qiacube and miRNeasy Mini Kit following the manufacturer's instructions (Qiagen, Hilden, Germany). Three sMSI-H and 11 SACs were included and hybridized using the Whole Human Genome Oligo Microarray® kit (Agilent) according to the purveyor instructions. Gene expression data were analyzed by Gene Set Enrichment Analysis (GSA) and different functional annotation databases (KEGG database and the Biological Process, Molecular Function and Cellular Component from Gene Ontology (GO). qPCRs were performed using SYBRgreen (Qiagen) in a 7500 Fast Real Time PCR system (Applied Biosystems).
Results: Differentially expressed functions included the KEGG pathways 'antigen processing and presentation', 'chemokine signaling pathway', 'cytokine–citokineR interaction' and 'TLR signaling pathway'. A total of 440 significantly upregulated and 532 downregulated genes were found in SAC. We selected *CRCP* and *CXCL14* genes as upregulated in SACs and *ICAM1* as upregulated in sMSI-H.
Validation by qPCR confirmed higher *ICAM1* expression in MSI-H (p = 0.0183) whilst *CRCP* and *CXCL14* expression showed the same trend as in the array but without reaching significance probably due to small sample size. ICAM1 protein expression by immunohistochemistry was also in agreement with microarray findings (preliminary data not shown).
Conclusions: These results point out that SAC and sporadic MSI-H CRC have dissimilar tumour biology features, immune-related being one of the most remarkable differentially expressed functions. Our findings suggest an important role of ICAM1 in immune response to MSI-H tumors and could partially explain why SACs seem to be less recognized by the immune system.
No conflict of interest.

897 Co-expression of chimeric antigen receptor (CAR) and miRNAs to T cell therapy

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Background: Advances in the use of cytotoxic T lymphocytes modified with chimeric antigen receptors (CARs) can be observed in clinical trials. Good therapeutic responses in hematological tumors have been described with this approach; although there are still field to optimize this response. Precursor B cell acute lymphoblastic leukemia (B-ALL) expressing the CD19 molecule has been clinically targeted by CARs. To increase the antitumor response we explored the association of anti-CD19−41BB-zeta CAR (α19BBζ) and microRNAs, described recently as modulators of T cell activation. In this context, miR-181a is an interesting candidate to co-expression with CARs, due to its role in regulating the mRNA expression of multiple phosphatases, leading to the reduction of the T cell receptor activation signaling threshold. miR-182, which has the ascribed function of increasing cell proliferation by targeting the transcription factor FoxO1, is another potential target to increase the capacity of T cells to expand upon CAR mediated activation due to antigen recognition. We aim co-express CARs and miR-181a or miR-182 in primary T lymphocytes in order to evaluate potential functional improvements in antitumor responses *in vitro*.
Material and Method: Peripheral blood mononuclear cells (PBMC's) from healthy donors were electroporated with bidirectional *Sleeping Beauty* transposon for simultaneous expression of the CAR and miRNA. Gene-modified T cells were expanded and activated *ex-vivo* by co-culture with irradiated L388 cell line. The expression of α19BBζ and miRNAs and targets were evaluated by FACS and RQ-PCR, respectively. *In vitro* expanded T lymphocytes were phenotyped for memory population, CD8 + and CD4 +, and evaluated for expression of activation markers and activity of lysis of target pre-B ALL cell line Nalm-6.
Results: The bidirectional system was efficient for the co-expression of α19BBζ and the miRNA in human T cell with efficiencies ranging from 30 to 50%. Stimulated T lymphocytes expanded vigorously *in vitro* with miRNAs groups expanding more than control groups (55 fold expansion for miR-181a and 12 fold expansion for miR-182).
Conclusions: The co-expression of miRNAs and CAR seems to impact the rate of T lymphocyte expansion for cells expressing CARs. We are currently expanding the number of donors evaluated and analyzing the impact of miRNA+CAR expression on the cytotoxic functions of T cells.
No conflict of interest.