Delivering complete responses against solid tumors by checkpoint blockade enabled with tumor necrosis factor alpha and interleukin-2 armed adenoviruses.

Cervera-Carrascon V, Siurala M, Santos JM, Havunen R, Sorsa S, Hemminki, A

Background

Immune checkpoint blockade became a revolutionary tool to unleash patient’s immune system against their malignancies. Even if the approach is highly promising, only a subset of patients take benefit from it leaving room for improvement. One of the biggest difficulties to make the therapy work appears when the immune system is not able to detect or penetrate the tumor. Oncolytic viruses are an attractive way to make immune checkpoint blockade work where in those “cold tumors” and to improve it where it works. Due to their nature, adenoviruses are recognized by different members of the innate defenses which will generate an increased attention from the immune system to the tumor. Furthermore, our viruses are engineered to express tumor necrosis factor alpha (TNFα) and interleukin-2 (IL-2), two cytokines to improve the trafficking to the tumor and enable T-cell mediated responses in the tumor.

Aims

• Assess how virotherapy affects the outcomes of the checkpoint blockade in both antitumor efficacy and overall survival.
• Study the changes produced in the tumor microenvironment coming after different treatments.
• Establish a rationale for choosing an administration sequence plan, regarding the timeframe of both immunotherapies.

Conclusions

• Virotherapy enables immune checkpoint blockade improving (p-value<0.00001) the antitumor effect.
• Triple therapy (ACT, immunecheckpoint blockade and virotherapy) display the best (p-value<0.05) antitumor efficacy.
• The strongest antitumor effect is also correlated with the strongest exhausted TCD8 profile.

Results

Figure 1 Antitumor efficacy of checkpoint blockade when combined Adoptive cell therapy and virotherapy. Different permutations of the three treatments led to an inconclusive idea that triple combination of the therapies is the best treatment.

Figure 2 Flow cytometric analyses of tumor samples reveal immune changes in the tumors. As described in figure 1A, 6 animals per group were killed and their tumors were analyzed to analyze different cell populations.

Figure 4 Individual lines of tumor growth after treating with checkpoint blockade, adoptive cell therapy and virotherapy. The experiment was repeated with larger amount of animals (n=84) to confirm which is the best group. Triple combination therapy displayed the best results (p-value<0.05).

Figure 5 Individual lines of tumor growth after treating with checkpoint blockade, adoptive cell therapy and virotherapy. Tumor samples from Fig.3 experiment were collected on day 13 after treatment. It is a correlation between antitumor efficacy and exhausted TIL status.

Figure 6 Analysis of systemic presence of tumor specific antigens. Spleens from 6 animals per group at day 11 and from survivors at day 90 were processed and analyzed to quantify different tumor specific responses. CD8 positive cells were found both B16.OVA specific antigens (ovalbumin) and wide spread melanoma antigens (Trp-2 and gp100).

We thank Eleonora Munaro, Susanna Grönberg-Vähä-Koskela and Minna Oksanen for their assistance during the study. The Biomedicum Flow Cytometry Core Facility (University of Helsinki) as well as the Laboratory Animal Centre (University of Helsinki) are appreciated for technical support. This study was supported by Marie Skłodowska-Curie Innovative Training Networks (ITN-EID VIRION H2020-MSCA-ITN-2014 project number 643130), Jane and Aatos Erkko Foundation, HELSING Research Funds (EY95), Sigrid Juselius Foundation, Finnish Cancer Organizations, University of Helsinki and TILT Biotherapeutics Ltd.

Corresponding author: Victor Cervera-Carrasco at victor@tiltbio.com

Acknowledgements