regrowth, suggesting the development of immunological memory in hetIL-15 treated mice. hetIL-15 promoted tumor accumulation of proliferating and cytotoxic CD8+ T and NK cells. Additionally, peritumoral hetIL-15 administration resulted in an increased tumor infiltration of both conventional type 1 dendritic cells (cDC1s) and of a novel DC population found only in the hetIL-15 treated animals. Phenotypic profile analysis confirmed the expression of several cDC1 specific markers, including CD103 and IRF8 on this DC population. Transcriptional and flow analysis of intratumoral dendritic cells indicate that the new hetIL-15 induced cells reside preferentially in the tumors and are distinct from cDC1 and cDC2 populations. Both cDC1s and the novel DC population were inversely correlated with the tumor size.

**Conclusions** Locoregional administration of hetIL-15 results in complete eradication of EO771 and significant reduction of 4T1 primary breast cancer tumors, prolonged survival and long-lasting specific anti-tumor immunity. hetIL-15 increases the tumor infiltration of activated T and NK cells and intensifies the tumor infiltration of conventional type 1 dendritic cells (cDC1) and a new population of dendritic cells. We propose that the anti-cancer activity of hetIL-15 in primary EO771 tumors is orchestrated by the interplay of NK, CD8+ T cells, cDC1 and a novel subset of DCs with a distinct phenotypic profile. These findings suggest a role for hetIL-15 in the treatment of breast cancer.

**Ethics Approval** The study was approved by the National Cancer Institute-Frederick Animal Care and Use Committee, approval number 19–324 and was conducted in accordance with the ACUC guidelines and the NIH Guide for the Care and Use of Laboratory Animals.

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**Abstracts**

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**576** NL-201, A DE NOVO IL-2 AND IL-15 AGONIST, DEMONSTRATES ENHANCED IN VIVO ANTITUMOR ACTIVITY IN COMBINATION WITH MULTIPLE CANCER IMMUNOTHERAPIES

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**Background** NL-201 is a de novo IL-2 and IL-15 receptor agonist designed with enhanced affinity for IL-2Rβγ and no binding interface for IL-2Rα (CD25). Previously, we reported that NL-201 stimulates selective proliferation of CD8+ effector T cells and NK cells, leading to increased CD8+ Treg and NK:Treg ratios in the tumor microenvironment. As a result, NL-201 treatment led to robust single-agent antitumor activity in syngeneic murine tumor models at well-tolerated doses.

**Methods** Here, we evaluated the antitumor activity of NL-201 in combination with established and exploratory cancer immunotherapies, including tumor-targeting monoclonal antibodies and immune checkpoint inhibitors (CPIs). Specifically, we evaluated NL-201 in combination with an anti-gp75 antibody (TA99) in a murine melanoma model, or anti-PD-1 and anti-PD-L1 antibodies in a CPI-resistant murine colon cancer model.

**Results** NL-201 synergizes with TA99, anti-PD-1, and anti-PD-L1 to inhibit tumor growth more effectively than either agent alone. The synergy of NL-201 with TA99 may result from enhanced NK-mediated antibody-dependent cellular cytotoxicity (ADCC), while the synergy with CPIs may result from CD8+ T cell stimulation, which can turn ‘cold’ tumor microenvironments ‘hot’.

**Conclusions** The broad activity of NL-201 across diverse tumor models and its potential to be combined with a variety of established and exploratory cancer immunotherapies to achieve synergistic antitumor activity highlights the opportunity for NL-201 to become a critical component of future immunotherapy regimens.

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**577** ENGINEERING NON-PATHOGENIC SYNTHETIC BIOTIC PRODUCING L-ARGININE SYNERGIZE WITH PD-1-BASED CANCER IMMUNOTHERAPY

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**Background** The availability of L-arginine in tumors is a key determinant of an efficient anti-tumor T cell response. Consequently, the elevation of typically low L-arginine levels within the tumor may greatly potentiate the anti-tumor responses of immune check point inhibitors, such as PD-L1 blocking antibodies. However, currently no means are available to locally increase intra-tumoral L-arginine levels.

**Methods** We used a synthetic biology approach to develop an engineered probiotic Escherichia coli Nissle 1917 strain that colonizes tumors and continuously converts ammonia, a metabolic waste product that accumulates in tumors, into L-arginine.

**Results** Colonization of tumors with these bacteria elevated intra-tumoral L-arginine concentrations, increased the amount of tumor-infiltrating T cells, and had striking synergistic effects with PD-L1 blocking antibodies in the clearance of tumors. The anti-tumor effect of the living therapeutic was mediated by L-arginine and was dependent on T cells.

**Conclusions** These results show that engineered microbial therapies enable metabolic modulation of the tumor microenvironment leading to enhanced efficacy of immunotherapies.

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**578** TUMOR SELECTIVE IMMUNE RESPONSES OF STA551, A NOVEL ANTI-CD137 AGONIST ANTIBODY ACTIVATED BY EXTRACELLULAR ATP

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**Background** Agonistic antibodies targeting CD137 in clinic have failed due to severe hepatotoxicity, leading to the development of bispecific approaches that must rely on high tumor-associated antigen expression to crosslink CD137. Here we report on STA551, a novel anti-CD137 agonist antibody which binds to CD137 only in the presence of ATP. Extracellular ATP concentration is well-known to be elevated in tumor tissue while remaining tightly regulated in non-tumor tissue, suggesting that STA551 can activate immune cells only in tumor tissue and not elsewhere. Thus, STA551 has great potential to overcome the limitations of conventional CD137-targeted antibodies.
LACTATE DEHYDROGENASE C-ASSOCIATED MOLECULAR NETWORKS PREDICT ENHANCED TUMOR GROWTH AND IMPAIRED IMMUNE RESPONSE IN BREAST CANCER

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Background Cancer testis antigens (CTAs) have gained interest in the field of anti-cancer therapy as they offer the opportunity to target tumor cells with little off/on-target side effects given their restricted expression patterns. Several CTAs have been implicated as mediators of cancer hallmarks including cancer metabolism, proliferation, survival, and cell motility. Lactate dehydrogenase C (LDHC) expression has been observed in various cancer types and likely confers a survival advantage to tumor cells through metabolic reprogramming. Thus, targeting LDHC has the potential to inhibit tumor growth and release the anti-tumor immune response from the acidic immunosuppressive microenvironment. This study aimed to explore the changes in the transcriptome of breast cancer cells upon in vitro LDHC targeting.

Methods We silenced LDHC expression in two breast cancer cell lines (BT549, HCC1954) and investigated the downstream effects on the tumor cell transcriptome. In addition, differentially expressed genes were subjected to regulatory network analyses and expression of key regulators was interrogated in the TCGA breast cancer dataset.

Results We identified 47 up- and 55 down-regulated transcripts after LDHC silencing (2.0-fold change, adj p<0.05). Specifically, we found that LDHC expressing breast cancer cells display an enrichment of genes involved in canonical pathways regulating cell cycle checkpoint control, BRCA1-mediated DNA damage response and NF-kb signaling in response to infection, which is in line with some of our unpublished work. In support, downstream effector analysis demonstrate that LDHC silencing negatively affects biological functions such as cellular development, cellular growth and proliferation, cell migration and cell infiltration. Upstream regulator analyses revealed that the observed changes in gene expression are associated with mTOR (p=1.27e-5, z=2.242) and CASP3 (p=3.2e-4, z=2.50) mechanistic networks, which in the presence of LDHC are predicted to activate TP53, Myc, NF-KB complex, STAT1/3, PRKCI, CDPK2, FOXO3 and HIF-1a while inhibiting SMAD3, PTEF, ATM, IL18 and BCL2. Furthermore, causal network analysis revealed a higher-level regulation by miR378a-3p (p=1.4e-7, z=-3.117), affecting the mechanistic networks and ultimately promoting tumor cell viability and proliferation, tumor cell movement and cell cycle progression in LDHC expressing cells. Interestingly, the miR378a causal network also indicated inhibition of the immune response in LDHC positive cells. Correlation analysis using the TCGA breast cancer dataset indicated a weak correlation between LDHC expression and the mechanistic regulator mTOR (R=0.26, p=1.82e-18).

Conclusions Our findings demonstrate that therapeutic targeting of LDHC may inhibit tumor growth while releasing the anti-tumor immune response in breast cancer, and warrant further in-depth investigation.

Acknowledgements This work was supported by a grant from the Qatar Biomedical Research Institute (grant number VR94), awarded to Dr Julie Decock.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0579

HIGH DOSE-RATE BRACHYTHERAPY OF LOCALIZED PROSTATE CANCER CONVERTS TUMORS FROM COLD TO HOT

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Background Prostate cancer is frequently cured with high dose-rate brachytherapy (HDRBT) radiation as a front-line treatment. Although considered to be an immune-excluded tissue, immune responses to radiation are implicated in driving tumour-eradication in prostate cancer.1 This has not been proven, and yet it is used as the rationale for clinical trials combining radiation and immunotherapies.2 We hypothesise that there is a predictable relationship between radiation and the immune responses in prostate cancer that could be used to provide sound rationale for specific immune interventions in solid tumours that are made possible by radiation therapy.

Methods We present here new results stemming from our recently published immunoprofiling study of world-unique pre- and post-radiation tissues from 24 prostate cancer patients (figure 1A), RadBank cohort.3 These samples were assessed using immune cell multiplex IHC, gene expression profiling, digital spatial profiling (DSP) and computational analysis of cell distribution.

Results This study unequivocally revealed that high dose-rate radiation converts predominately ‘cold’ prostate tumour tissue