Cryo-Immune Vaccination (CIV) by SYNC-T therapy: Preclinical modeling of a novel device-multidrug immunotherapeutic approach to eradicate advanced metastatic cancers

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Abstract

Advanced cancers characterized by fulminant metastasis and drug resistance remain the top challenge in cancer research. Here we introduce a new general approach to this challenge termed **Cryo-Immune Vaccination (CIV)**. Briefly, CIV involves several treatment cycles comprised of (1) cryo-lysis of a single lesion in a metastatic subject, to generate an *in situ* personalized vaccine at each cycle, plus (2) locoregional infusion of the cryo-lysed lesions with a low-dose cocktail of multiple immunotherapeutic drugs. This type of device/drug-generated cancer vaccine has never been explored methodologically. **Notably, positive systemic responses to this locoregional treatment are being observed in Phase 1 studies of SYNC-T therapy, the CIV lead approach. Accordingly, there is an urgent need of preclinical models to investigate the basis for CIV safety, efficacy and mechanisms of action.** Here we report the creation of a mouse model of CIV treatment, adapting the metastatic murine breast tumor 4T1 to study allometrically scaled doses of SYNC-T therapies in clinical development by Syncromune Inc. (i.e., SV-101 and SV-102). **Our work establishes an initial model of CIV treatment for metastatic solid tumors, and it suggests that CIV may promote survival by a process that relies upon IDO1 blockade.**

Background

CHALLENGES:

Cancer immunotherapy outcomes might be improved in advanced cancer patients by combining personalized neoantigen vaccines with higher-order combinations of immunotherapy drugs (>2 drugs). However, key questions exist:

(1) Which neoantigens & adjuvants in vaccines? Many uncertainties and complexities exist in the choices needed to elicit effective response in different patients[1,2].

(2) Where and how to deliver vaccines? Immune context within and proximal to the TME may be important to guide effective neoantigen and adjuvant responses[3,4].

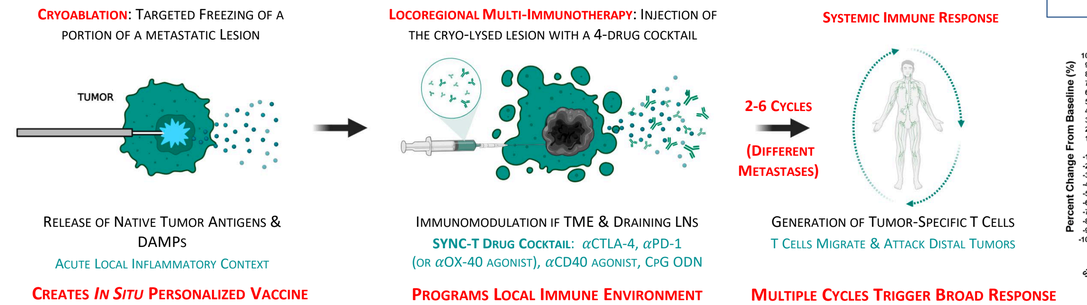
(3) How to bypass toxicities of multi-API immunotherapy (i.e. >2 drugs)? Systemic administration of higher-order ICT causes acute autoimmune reactions.[5]

PROPOSED SOLUTIONS:

(1) Tumor cryoablation to create intratumoral waves of native (non-denatured) neoantigens in a highly inflammatory adjuvant setting ('hypothermic frostbite'). Tumor cryoablation exerts positive immunogenic effects, including at distal untreated lesions[6,7]. Each cycle of cryoablation of a metastatic lesion generates neoantigens presented in a local immune context ('lesion-personalized vaccine').

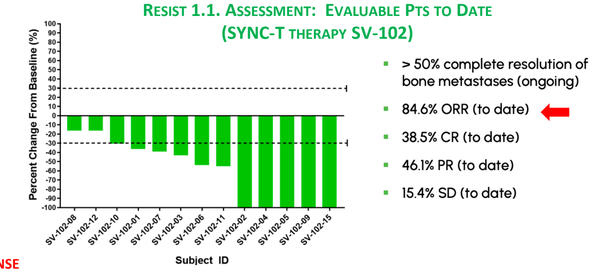
(2) Locoregional inection of higher-order immunotherapy combinations (>2 drugs) to improve immune context and limit autoimmune toxicities of systemic delivery. Locoregional ICT can trigger systemic antitumor immune responses[3,4]. Each cycle of CIV includes an intratumoral injection of a low-dose immunotherapy 'cocktail' into the cryo-lysed lesion, relieving local immune suppression while limiting systemic autoimmune attacks.

Overview of Cryo-Immune Vaccination with SYNC-T Therapy



Phase 1 Data: Metastatic CRPC Pts

(Link CJ et al., AACR 2024 Abstract Presentation)



Results

Fig. 1. BALB/c host survival after orthotopic engraftment of 4T1 BRCA cells. Primary tumors were initiated by injection of mammary fat pads (1x10^4 cells). Lung metastases seeded rapidly in this aggressive model cause death. *Ido1*$^{-/-}$ mice survive longer than WT mice but eventually succumb to death due to lung metastases[8].

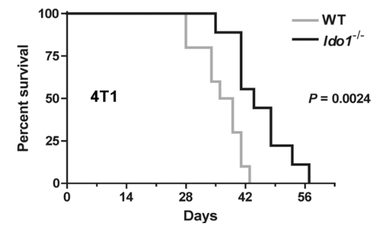
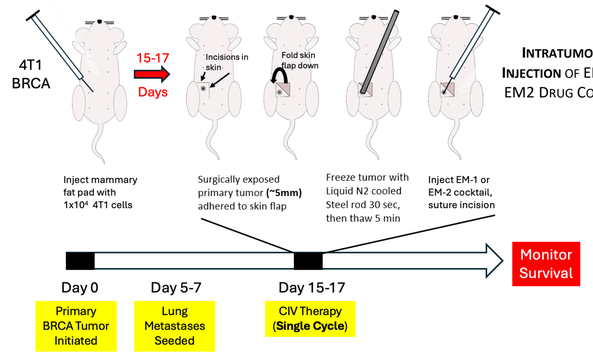


Fig. 2. CIV Treatment Design. Iceball formed by liqN2-cooled probe partially ablates tumor (50-70%). Doses of immunotherapy drugs (Invivogen) were allometrically scaled human to mouse and injected in a single bolus of 25 µl into the cryolysis-cleared gel-like part of the tumor.

EM1 cocktail = murine αCTLA-4, αOX-40 agonist, αCD40 agonist, CpG ODN (50µg each)
= SYNC-T human therapy SV-101

EM2 cocktail = murine αCTLA-4, αPD-1, αCD40 agonist, CpG ODN
= SYNC-T human therapy SV-102



Intratumoral Injection of EM1 or EM2 Drug Cocktail

Fig. 3. CIV does not affect survival in WT hosts engrafted with 4T1 BRCA. Results reflect the mean survival from two trials of each therapy (n=5 subjects per cohort).

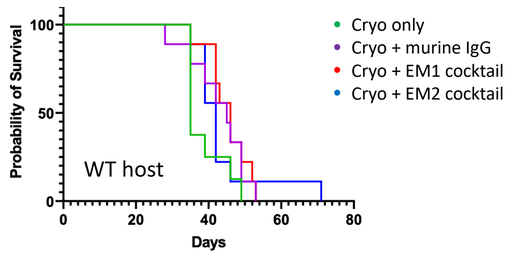
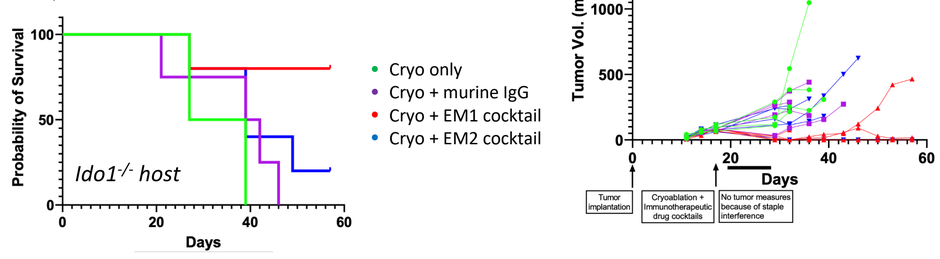


Fig. 4. CIV promotes survival in *Ido1*$^{-/-}$ hosts engrafted with 4T1 BRCA. Results reflect the survival from one trial of each therapy (n=5 subjects per cohort).



Methods and Materials

Murine BALB/c 4T1 breast tumor graft model. 4T1 is an aggressive orthotopic breast tumor that metastasizes to lung, bone and brain. To initiate tumors, naïve immunocompetent BALB/c hosts were engrafted with 1x10^4 4T1 cells by injection into mammary fad pads. **Tumor cryoablation.** When primary tumors reached ~5-7 mm in largest extent, a time when occult pulmonary metastases are already seeded[8], they were exposed surgically on skin flaps and contacted directly with a liquid nitrogen-cooled blunt-pointed 8 mm steel rod for 30 sec until a visual iceball was observed. The goal was to produce a partial ablation of ~80% of the tumor. The rod was then withdrawn and tumor tissue allowed to thaw fully (~5 min) as monitored visually and by re-warming of the external side of the skin flap by touch. **Intratumoral drug injection.** Cryo-lysed primary tumors were administered one (1) cycle of a multidrug cocktail (or negative control murine IgG) in a total volume of 25 µl. Drugs matched those in SYNC-T therapies SV-101 and SV-102 presently in human trials (Syncromune, Inc., including murine-reactive mAbs against CTLA4, PD-1 **or** OX40, CD40L and CpG-ODN (Invivogen). Doses were allometrically scaled to SV-101 and SV-102 (Syncromune, Inc.). Skin flaps were sutured and subject survival was monitored post-operatively[8].

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