Preclinical Efficacy of Chemotherapeutic Agents in Combination with Immune checkpoint Inhibitors in Syngeneic Tumor Models

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ABSTRACT

INTRODUCTION AND BACKGROUND: Immunotherapy has transformed cancer care with promising strategy for controlling tumor growth and metastatic spread with significant advancement in patient outcomes. Over the past few years, immunotherapy has evolved as one of the potential and practical therapeutic options for primary and metastatic tumours. Preclinical evaluation of chemo agents in combination with immune check point inhibitors has not been thoroughly investigated in murine models.

METHODS: In the present study, we have evaluated the preclinical efficacy of conventional chemo agents Cisplatin, Doxorubicin as standalone and in combination with anti-PD1 & anti-CTLA4 in four different syngeneic tumor models [Murine colon tumor - MC38, CT26, Lewis lung carcinoma (LLC), Breast - 4T1] in immunocompetent mice. Mice were randomized based on tumor volume (~100mm^3) and treatment was initiated with Cisplatin – 5mg/kg, i.v; Q5D x 4 doses or Doxorubicin – 4mg/kg, i.v; Q4Dx 4 doses as standalone or in combination with anti-PD1/anti-CTLA4 at 10mg/kg, ip; Q4D x 4 doses. Tumor volume, changes in body weight, clinicals signs, mortality were monitored twice weekly upto 3 weeks. Experiment was completed on day 21 and all mice were humanely euthanized.

RESULTS: In MC38 tumor model, standalone treatment of Cisplatin and anti-PD1 resulted in mild to moderate tumor growth inhibition whereas anti-CTLA4 treatment resulted in poor efficacy. However, combination of Cisplatin with anti-PD1 had improved efficacy outcome when compared in combination with anti-CTLA4. In CT-26 tumor model standalone treatment with Cisplatin showed moderate efficacy whereas anti-PD1 and anti-CTLA4 exhibited mild to moderate tumor growth inhibition. Combination of Cisplatin + anti-PD1 treatment group showed high tumor growth inhibition compared to combination of cisplatin + anti-CTLA4 group. In Lewis lung carcinoma (LLC), standalone IO agent anti-CTLA4 and anti-PD1 did not show tumor growth inhibition, whereas conventional treatment with cisplatin and doxorubicin showed significant tumor growth inhibition. However, Cisplatin in combination with anti-PD1 showed highest tumor growth inhibition. In 4T1 breast tumor model standalone Doxorubicin, anti-CTLA4 and anti-PD1 showed statistically non-significant tumor growth inhibition whereas Doxorubicin in combination with anti-CTLA4 and anti-PD1 showed minimal tumor growth inhibition.

RESULTS – *IN VIVO* MONITORING OF ANTI-TUMOR RESPONSE IN MURINE SUBCUTANEOUS TUMOR MODELS

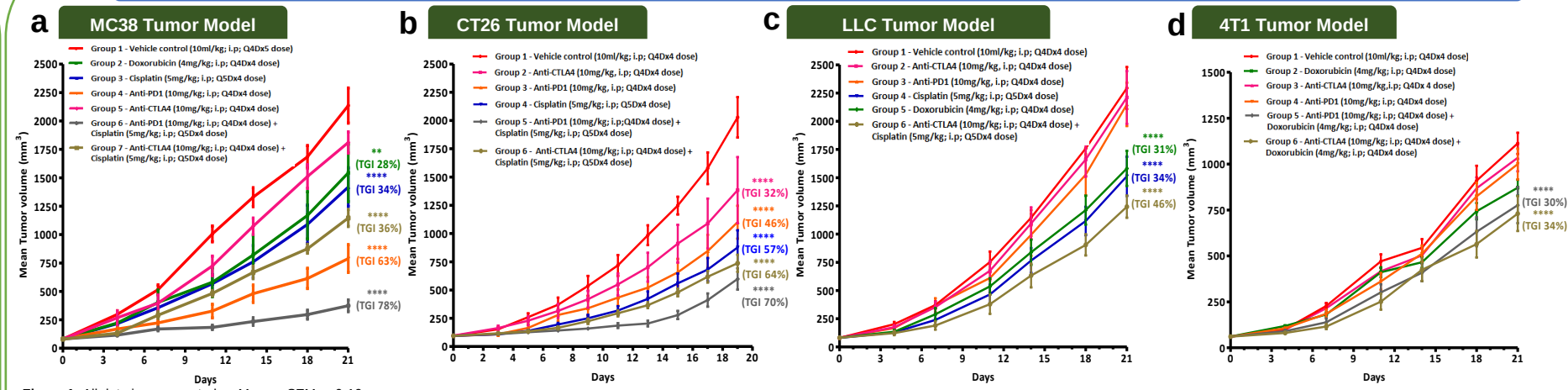
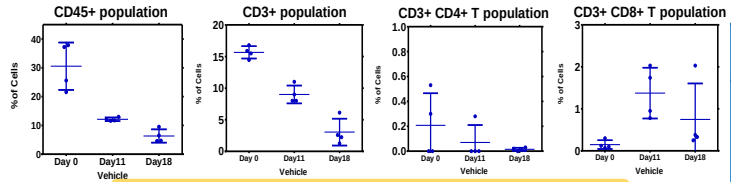


Figure 1: All data is represented as Mean ± SEM n=6-10
(a) In MC38 syngeneic tumor model standalone treatment of anti-PD1 resulted moderate tumor growth inhibition. However, in combination of Cisplatin with anti-PD1 had improved efficacy outcome when compared in combination with anti-CTLA4 **(b)** In CT-26 tumor model standalone treatment with anti-PD1 resulted in moderate tumor growth inhibition. Combination of cisplatin with anti-PD1 & anti-CTLA4 showed better tumor growth inhibition **(C)** In Lewis lung carcinoma (LLC) model, standalone conventional treatment with cisplatin and doxorubicin showed mild tumor growth inhibition. However, combination of Cisplatin with anti-PD1 resulted in better tumor growth inhibition **(d)** In 4T1 breast tumor model combination of Doxorubicin with anti-CTLA4 and anti-PD1 showed minimal tumor growth inhibition.
** p<0.01, **** p<0.0001 Statistically significant when respective treatment groups were compared with the respective control group.

Tumor immunoprofiling

Baseline TIL analysis in CT26 tumors (Day 0, Day 11 & Day 18)



Post treatment TIL analysis in CT26 tumors

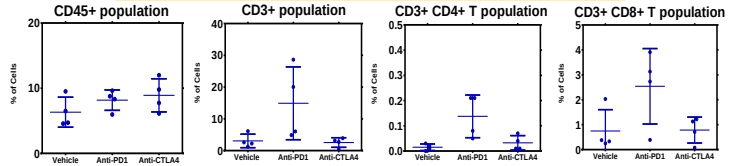


Figure 2: Multiparametric flow cytometry based analysis of the Lymphoid and Myeloid components infiltrating vehicle (n=5), anti-PDL1 (n=5) and anti-CTLA4 treated CT26 tumor.

Immune-profiling, IHC, Cytokine analysis

Sample	Immune Profile (Tumor & Blood)	IHC (Tumor)	Cytokine (Plasma)
Tumor Blood Plasma	**T cell:** CD45, CD3, CD4, CD8 **Treg:** CD4+ CD25+ FOXP3+ **B cell:** CD19, CD20 **NK cell:** CD16, CD56 **TAM:** CD33, CD11b, F4/80, Ly6C **MDSC:** CD33, CD11b, Ly6G **DC:** CD11c+ CD103+ CD24+	CD4 CD8 CD25 FOXP3	IFN-α, IFN-γ IL-1 beta IL-2, IL-10 IL-12/IL-23p4 IL-4, IL-5 IL-6 IL-8 (CXCL8) TNF-α GM-CSF Il-13 TNF beta

The above immune profiling, cytokine panel and IHC services are offered as per project needs.

CONCLUSIONS

- Altogether, the obtained data demonstrated the preclinical efficacy pattern of conventional chemo agents in combination with immune check point inhibitors in syngeneic tumor models.
- The degree of efficacy ranking for combination of chemo agents with anti-PD1/anti-CTL4 is MC38 > CT26 > LLC > 4T1.
- Further mechanistic studies are required to understand the efficacy in preclinical models and might pave road for potential clinical evaluation of combination treatment in patients.

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