Profiling of syngeneic models by anti-PD1, PD-L1 and CTLA-4 immunotherapeutics and RNAseq analysis provides predictive biomarkers for cancer immunotherapy

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Introduction

Background: Syngeneic tumor models have long been used in cancer research. The recent clinical success of anti-CTLA-4 and anti-PD1 antibodies contributed to increasing the interest around the use of syngeneic models to evaluate cancer immunotherapy. Surprisingly, although they were initially thought to be immunosuppressive, classic anticancer therapies, such as chemotherapy, targeted agents or radiotherapy, can promote antitumor immunity, thus synergizing with cancer immunotherapies. Suitable models in which to evaluate combination therapies are in great demand.

Importantly, in the clinic it is still unknown why some patients respond to certain immunotherapies while others do not. We set out to utilize syngenetic models to address these questions.

Material and methods: Syngeneic cell lines models, such as B16, CT26, MC38, 4T1, were used to evaluate the efficacy of anti-PD1, PD-L1, and CTLA-4 antibodies. Tumors were collected and RNAseq was performed to identify biomarkers predictive of response.

Results: Crown has established a large collection of syngeneic models that covers most tumor types. Our models have been extensively profiled *in vivo* using anti-PD1, anti-PD-L1, and anti-CTLA-4 antibodies, providing necessary information for models selection and dosing for combination therapy. Mostly recently, we have generated detailed expression maps and mutational profiles for our syngeneic models, and we have identified transcripts from alternative gene spliced, and gene fusion using RNAseq. Our analysis indicated that a number of syngeneic models harbor mutations that may sensitize them to combination strategies of targeted agents and immunotherapy.

Using proprietary genetic signature algorithms, we have also identified in our mouse models a set of biomarkers that may be useful to predict response to different type of immunotherapies.

Conclusions: These data will enable researchers to select the appropriate model for combination studies with immunotherapy, based on the expression of specific targets. In addition, biomarkers of response identified using our preclinical models can be used in predicting patient response in the clinic.

Methods

Animals and syngeneic models

Immunocompetent mice (such as C57BL/6, BALB/c or C3H) were used to generate syngeneic models. Mice were inoculates at right lower flank with 0.1ml suspension of tumor cell in PBS

Therapeutic

The treatments for the therapeutic study were started when mean tumor size reached 80-120 mm^3. Each group contained 6-10 tumor bearing mice.

Endpoints

Tumor volume was calculated using the formula: $V(mm^3) = (D \times d^2)/2$, where D and d are the long and short diameters of the tumor, respectively. The tumor size was then used to calculate tumor growth inhibition (TGI). Tumors were collected for FACS and RNAseq analysis.

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Results

Figure 1. Efficacy Evaluation of anti-PD1 (RMP1-14), anti-PD-L1 (10F.9G2), and anti-CTLA-4 (9D9) in the Treatment of Murine Syngeneic Models

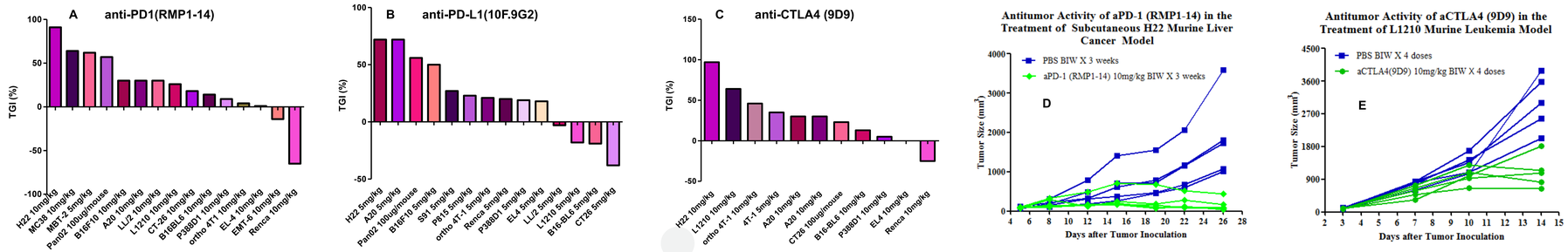


Figure 2. Sub-populations of Immune Cells in Syngeneic Tumors

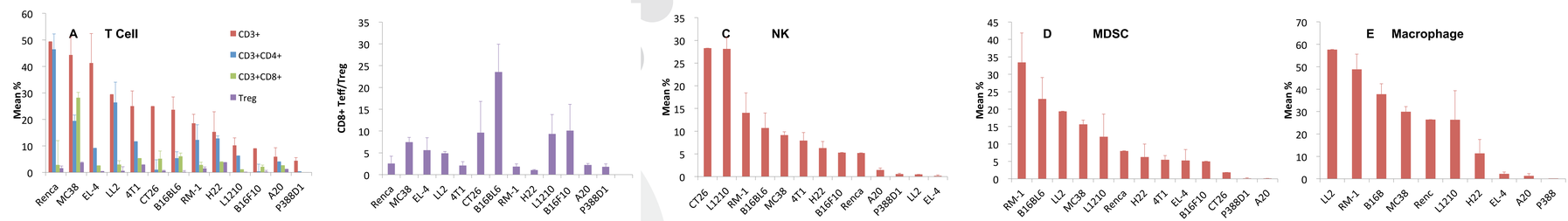


Figure 3. Hot Spot Mutations in Murine Tumors

MODEL	GENE	TRANSCRIPT	mRNA_POS	CDS_POS	REF	ALT	DEPTH(REF,ALT)	AMINO_ACID_CHANGE	CONFIDENCE	MUTATIONTYPE	Human Gene	human protein ID	Human Gene Position
B16BL6	Braf	ENSMUST00000102487	958	790	T	C	27:16	C264R	PASS	Missensemutation	BRAF	ENSP00000288602	C280
MC38	Braf	ENSMUST00000102487	1629	1461	G	T	18:17	W487C	PASS	Missensemutation	BRAF	ENSP00000288602	W450
MC38	Egfr	ENSMUST00000120329	3579	3307	G	T	24:5	G1103C	PASS	Missensemutation	EGFR	ENSP00000275493	G1103
A20	Erbb2	ENSMUST00000058295	1251	1058	G	T	0:2	G353V	PASS	Missensemutation	ERBB2	ENSP00000269571	E352
H22	Erbb2	ENSMUST00000058295	3426	3233	C	T	45:10	P1078L	PASS	Missensemutation	ERBB2	ENSP00000269571	P1077
L1210	Erbb2	ENSMUST00000058295	581	388	G	A	0:2	A130T	PASS	Missensemutation	ERBB2	ENSP00000269571	V129
CT26	Fgfr1	ENSMUST00000084027	1046	320	C	T	99:16	S107F	QD	Missensemutation	FGFR1	ENSP00000393312	S140
MC38	Fgfr3	ENSMUST00000114411	1741	1433	G	T	2:6	C478F	PASS	Missensemutation	FGFR3	ENSP00000339824	F485
L1210	Fgfr4	ENSMUST00000050452	2517	2177	C	T	0:2	A726V	PASS	Missensemutation	FGFR4	ENSP00000292408	A729
4T1	Flt3	ENSMUST00000049324	3213	2989	G	A	0:6	G997R	PASS	Missensemutation	FLT3	ENSP00000241453	-
4T1	Flt3	ENSMUST00000049324	2503	2279	T	C	0:6	L760S	PASS	Missensemutation	FLT3	ENSP00000241453	S759
4T1	Flt3	ENSMUST00000049324	423	199	G	A	0:8	A67T	PASS	Missensemutation	FLT3	ENSP00000241453	A66
A20	Flt3	ENSMUST00000049324	3213	2989	G	A	0:134	G997R	PASS	Missensemutation	FLT3	ENSP00000241453	-
A20	Flt3	ENSMUST00000049324	2503	2279	T	C	0:172	L760S	PASS	Missensemutation	FLT3	ENSP00000241453	S759
A20	Flt3	ENSMUST00000049324	423	199	G	A	0:23	A67T	PASS	Missensemutation	FLT3	ENSP00000241453	A66
B16BL6	Flt3	ENSMUST00000049324	2110	1886	C	T	0:2	T629M	PASS	Missensemutation	FLT3	ENSP00000241453	T628
CT26	Flt3	ENSMUST00000049324	3213	2989	G	A	0:2	G997R	PASS	Missensemutation	FLT3	ENSP00000241453	-
H22	Flt3	ENSMUST00000049324	3213	2989	G	A	0:2	G997R	PASS	Missensemutation	FLT3	ENSP00000241453	-
H22	Flt3	ENSMUST00000049324	2503	2279	T	C	0:2	L760S	PASS	Missensemutation	FLT3	ENSP00000241453	S759
H22	Flt3	ENSMUST00000049324	423	199	G	A	0:4	A67T	PASS	Missensemutation	FLT3	ENSP00000241453	A66

Figure 4. Prediction of Efficacy with Gene Expression Signatures

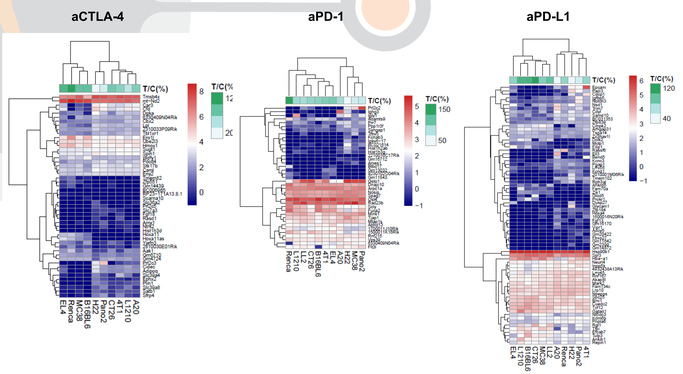


Figure 5. Mutation Load, DNA Repair Gene Mutations May not Predict Efficacy

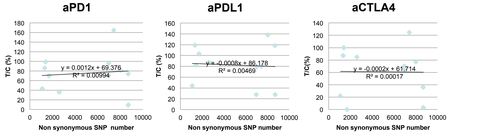
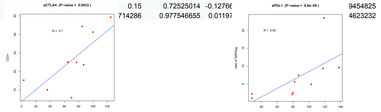


Figure 6. Base Line Immune Cell Population May Predict Efficacy

FACS	aCTLA4 pvalue	aCTLA4 cor	aPD1 pvalue	aPD1 cor	aPDL1 pvalue	aPDL1 cor
CD3+	0.043253968	0.7	0.22755151	0.419454825	0.82820248	0.079027721
CD3+CD4+	0.643639771	0.183333333	0.973408064	-0.012158111	0.373561333	-0.316110883
CD3+CD8+	0.775628307	0.116666667	0.578406145	-0.200608529	0.614067751	0.182371663
Treg	0.743540564	-0.133333333	0.326657258	-0.346500616	0.069155844	-0.595747433
ratio of Teff/Treg	0.3125	0.383333333	0.626123419	0.176292608	9.60E-05	0.930095482
NK	0.462990052	-0.283333333	0.789234533	-0.097264887	0.072785937	0.589668377
MDSC	0.15	0.72525014	-0.12767	9454825		
Macrophage	714286	0.977546655	0.01197	4623232		



Summary

- We established the antitumor activity of anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies and the basal levels of infiltrate immune cells from different lineages in a panel of syngeneic tumor models
- Gene profiling data can guide model selection and rational design of combination therapy
- A panel of genes were identified as potential predictive markers for immunotherapy in syngeneic models. Base line T cells and Teff/Treg ratio may predict efficacy of anti-CTLA-4 and anti-PD-L1, respectively