Profiling of Syngeneic Models by Check Point Inhibitors, RNAseq, and FACS Analysis Enables Better Selection of Models for Immune Targeted Combination Therapy

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Introduction

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 syngeneic models to evaluate cancer immunotherapy. Surprisingly, although they were initially thought to be immunosuppressive, classic anticancer therapies, such as chemotherapy or radiotherapy, can promote antitumor immunity, thus synergizing with cancer immunotherapies. Suitable models in which to evaluate combination therapies are in great demand.

To meet this demand, Crown Bioscience has established a large collection of syngeneic models that covers most tumor types. Our syngeneics have been extensively profiled *in vivo* using anti-PD1, anti-PD-L1, and anti-CTLA-4 antibodies, providing necessary information for selecting the appropriate models and doses for combination therapy. Most recently, we have generated detailed gene expression and mutation profiles for our models, as well as performed RNAseq to identify transcripts from alternative gene splicing, post-transcriptional modifications, and gene fusion. Moreover, our FACS analysis to isolate subpopulation of T cells, such as effector and regulatory T cells, provides insights about each checkpoint inhibitor's effect on immune cells.

Combining the *in vivo* immunotherapy profiles of our syngeneic models with comprehensive profiling data will enable models selection based on specific targets and the development of combination therapies that may in the near future benefit patients.

Methods

Animals and syngeneic models

Immunocompetent mice (e.g. C57BL/6, BALB/c or C3H) were used to generate syngeneic models. A suspension of tumor cells in 0.1ml PBS was inoculated in the right lower flank of each mouse.

Procedures

Treatment with the immunotherapeutic antibodies were started when mean tumor size reached 80-120 mm^3. 6-10 tumor bearing mice were included in each group.

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 is then used to calculate the TGI (tumor growth inhibition) values. Tumor samples were collected for FACS, IHC, IF and RNAseq analysis.

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Results

Figure 1. Crown Bioscience Mouse Syngeneic Models

Cancer Type	Cell lines	aPD-1	aPD-L1	aCTLA-4	RNAseq	TIL Profiling
Bladder	MBT-2	✓	ongoing	ongoing	ongoing	ongoing
Breast cancer	EMT-6	✓	ongoing	ongoing	✓	ongoing
	4T1	✓	✓	✓	✓	ongoing
Colon cancer	CT-26	✓	✓	✓	✓	✓
	MC38	✓	✓	✓	✓	✓
Kidney	Renca	✓	✓	✓	✓	ongoing
Leukemia	L1210	✓	✓	✓	✓	✓
Liver cancer	H22	✓	✓	✓	✓	ongoing
Lung cancer	LL/2	✓	✓	ongoing	✓	ongoing
Lymphoma	A20	✓	✓	✓	✓	✓
	P388D1	✓	✓	✓	✓	ongoing
	EL-4	✓	✓	✓	✓	ongoing
Melanoma	B16BL6	✓	✓	✓	✓	ongoing
	B16F10	✓	✓	✓	✓	ongoing
Pancreatic	Pan02	✓	✓	✓	✓	ongoing
Prostatic	RM-1	✓	✓	✓	✓	✓
B lymphocyte	BCL1 clone 5B1b			ongoing		
Breast	JC			ongoing		
Colon	Colon-26			ongoing		
Fibrosarcoma	WEHI-164			ongoing		
Leukemia	C1498			ongoing		
Lymphoma	L5178-R			ongoing		
	E. G7-OVA			ongoing		
Myeloma	MPC-11			ongoing		
Neuroblastoma	Neuro-2a			ongoing		
Plasmacytoma	J558			ongoing		
NSCLC	KLN 205			ongoing		

Figure 2. Efficacy of anti-PD1 (RMP1-14), anti-PD-L1 (10F.9G2) and anti-CTLA-4 (9D9) Treatment

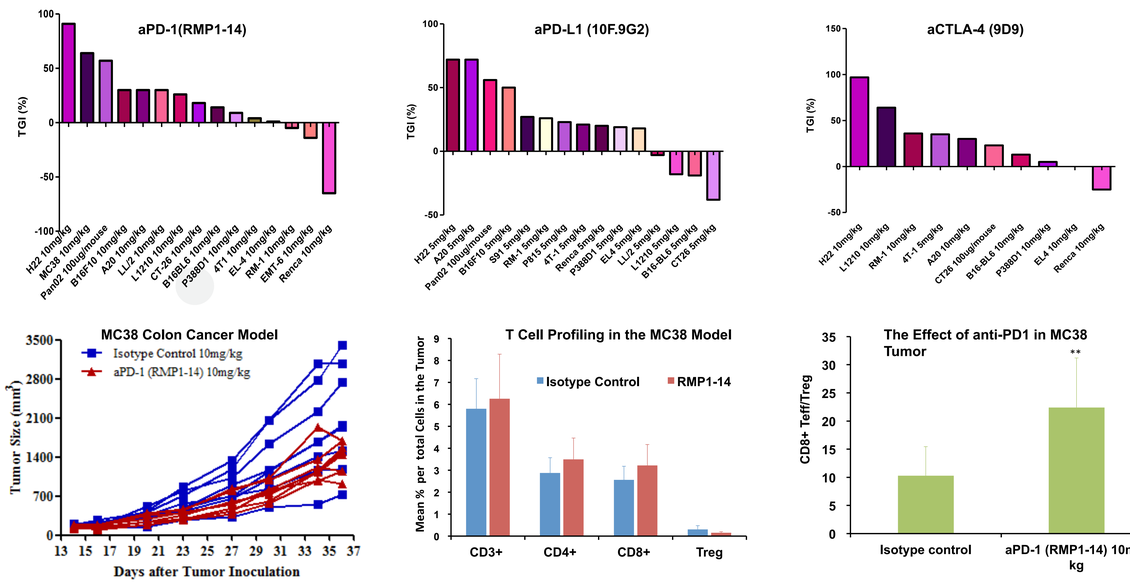

Figure 3. Immunological Profile by IHC and IF Analysis of the A20 Lymphoma Model

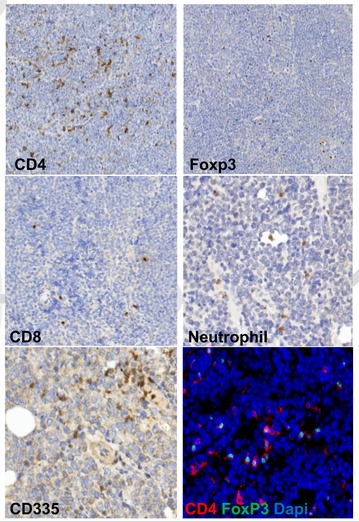

Figure 4. Tumor Driving Gene Expression in Murine Tumors

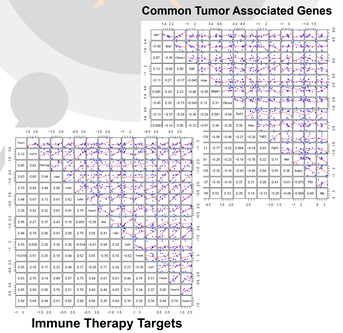

Figure 5. Hot Spot Mutations in Murine Tumors (partial list)

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	ENSMUST00000002487	958	790	T	C	27:16	C264R	PASS	Missensemutation	BRAF	ENSP00000288602	C280
MC38	Braf	ENSMUST00000002487	1629	1461	G	T	18:17	W487C	PASS	Missensemutation	BRAF	ENSP00000288602	W450
MC38	Egfr	ENSMUST00000030329	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	681	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	Fgfr3	ENSMUST00000005452	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 6. Whole Genome Gene Fusion Analysis in the 4T1 Model

up_gene	up_chr	up_strand	up_Genome_pos	up_loc	dw_gene	dw_chr	dw_strand	dw_Genome_pos	dw_loc	Span_reads_num	Junc_reads_num	Fusion_Type	down_fusion_part_frame_shift_or_not
Cbfa2b	chr13	-	60997599	E	4930486L24Rik	chr13	-	60956310	E	3	16	INTRACHR-SS-OGO-0GAP	frame-shift
D17H6S56E-5	chr17	-	35137158	M	Errfi1	chr4	+	1.5E+08	M	2	2	INTERCHR-DS	NA
Eapp	chr12	-	55774558	M	1110002B05Rik	chr12	-	55747522	E	2	2	INTRACHR-SS-OGO-1GAP	NA
F630111L10Rik	chr3	-	58957341	M	P2ry14	chr3	-	58920213	E	8	10	INTRACHR-SS-OGO-0GAP	NA
H2-M3	chr17	+	37410855	M	Olfr755-ps1	chr17	+	37414914	M	2	5	INTRACHR-SS-OGO-0GAP	NA
Klhdc5	chr6	+	1.47E+08	M	Paip2b	chr6	-	83763047	M	2	6	INTRACHR-DS	NA
Knlc1	chr5	+	1.24E+08	E	Snrnp35	chr5	+	1.25E+08	E	3	11	INTRACHR-SS-OGO-1GAP	frame-shift
Ptms	chr6	-	1.25E+08	M	Ptma	chr1	+	88426037	M	2	2	INTERCHR-DS	inframe-shift
Sema4d	chr13	-	51800322	E	Gm15440	chr13	-	51796409	M	2	4	INTRACHR-SS-OGO-0GAP	inframe-shift
Vav3	chr3	+	1.09E+08	E	Chst7	chrX	+	19674091	E	1	1	INTERCHR-SS	NA
Znrf1	chr8	+	1.14E+08	E	Zfp1	chr8	+	1.14E+08	E	1	1	INTRACHR-SS-OGO-0GAP	NA

Summary

- The efficacy of anti-PD1, anti-PD-L1 and anti-CTLA-4 antibodies as anticancer agents was established in a panel of syngeneic tumor models;
- FACS, IHC and IF analysis are useful tools in illustrating the role played by different immune cell populations in response to anticancer agents;
- The gene profiling data will guide informative selection of models and a more rational design of immune-targeted combination therapy.