CrownBio
CONNECTING SCIENCE TO PATIENTS

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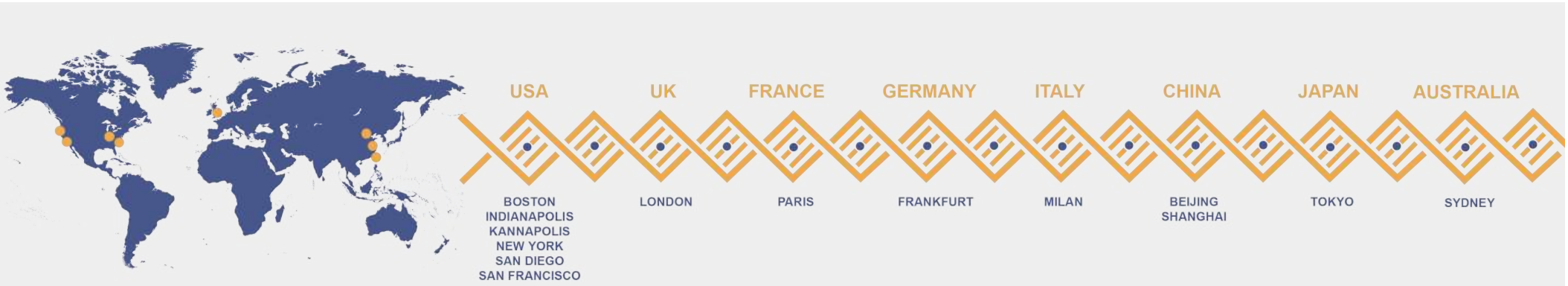
Tumour heterogeneity and combination strategies

Rajendra Kumari, CBUK Ltd



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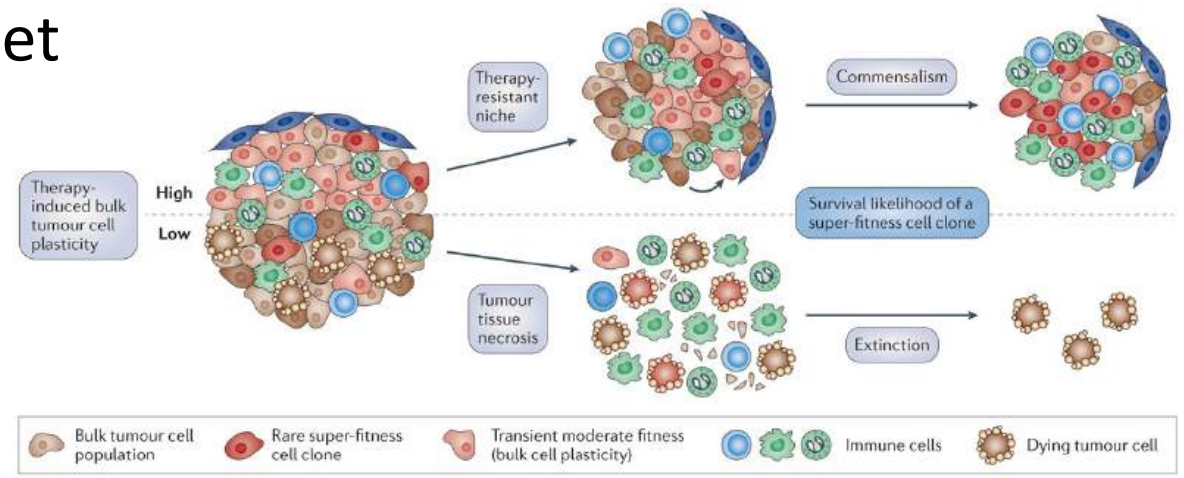
Contents

- Tumour antigen heterogeneity and mutational load influences antitumour immune response
- Importance of tumour antigens in triggering antitumour immune response
- Immuno phenotyping of mouse models
- Human target

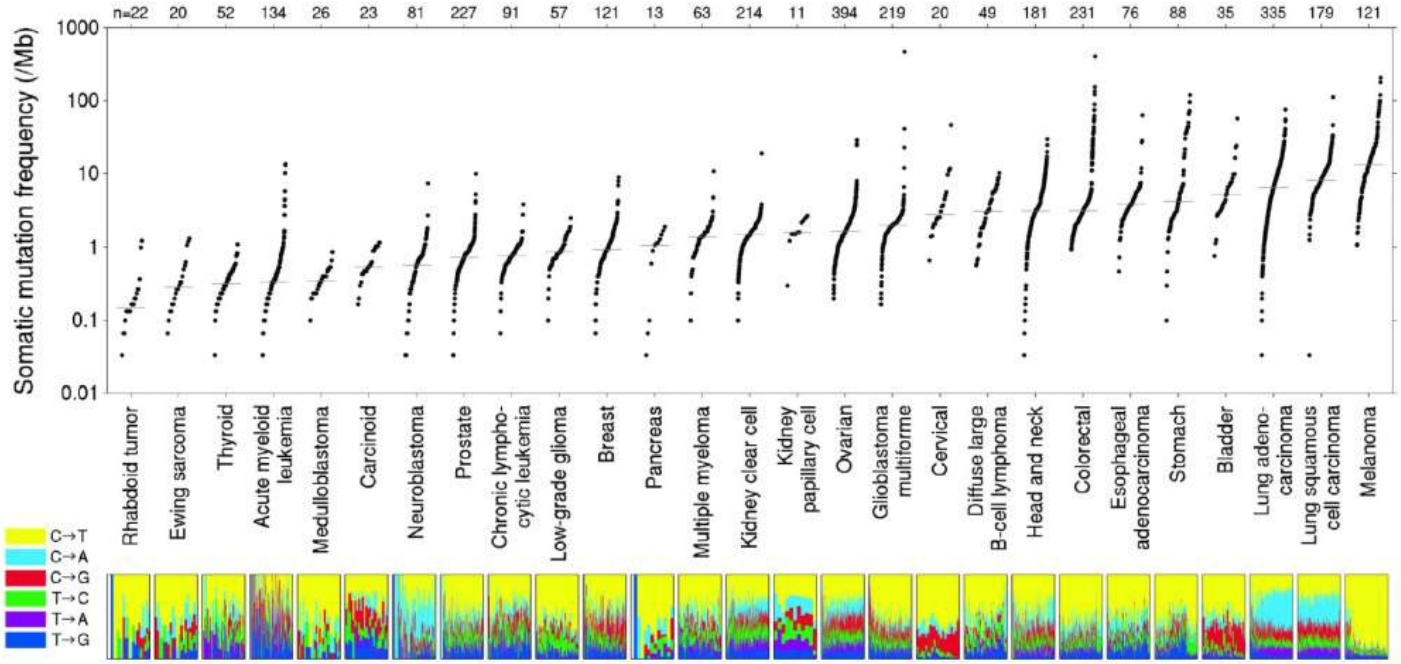


Nature Reviews | Cancer

Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance?
Michael Hölzel, Anton Bovier & Thomas Tüting. Nature Reviews Cancer 13, 365-376 (May 2013)

Mutational heterogeneity in cancer

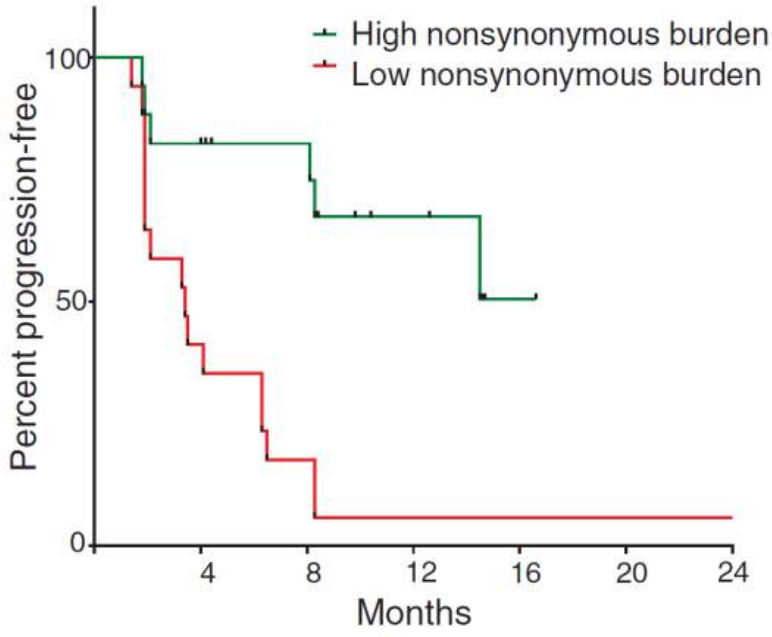
- Neoantigens: nonsynonymous somatic variations acquired by tumours
- Tumours with mutational burden potentially harbour immunogenic antigens resulting in antigens that trigger an anti-tumour immune response e.g. melanoma and lung
- Carcinogens e.g. tabacco, UV and DNA mismatch repair have high mutations
- Establish predictive biomarkers

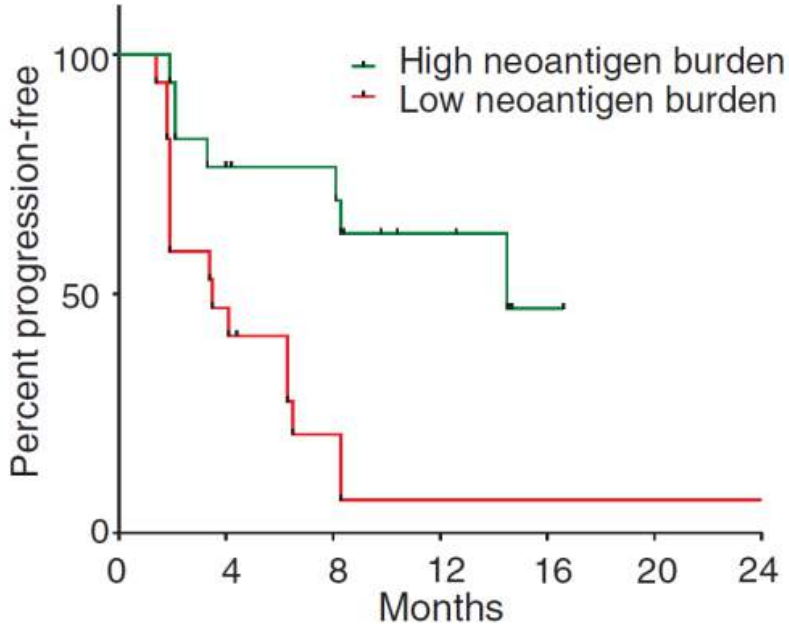


Lawrence et al; Nature. 2013 July 11; 499(7457):

Mutation mediated sensitivity to PD-1 blockade in NSCLC

- Correlation between neoantigens and mutations
- Both high nonsynonymous mutational and neoantigen burdens are associated with antitumour immune response and clinical response to checkpoint inhibitors
- Genomic landscape shapes the response to anti-PD-1 therapy





Rizvi et al Science 2015, 348: 124-128

Inducing Immunogenicity

- High burden of clonal neoantigens (i.e. present in all tumour cells) is associated with improved patient survival and durable response to immunotherapy *(Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. McGranahan Science 2016)*
- Most mutations however occur early on
- Treatment of cancer can induce antigen expression *(Combining immunotherapy and targeted therapies in cancer treatment. Vanneman & Dranoff, Nature Rev Cancer 2013, 12:237-251)*
- Induction of immunogenic cell death can increase antigen presentation to T cells *(Immunogenic Cell Death in Cancer Therapy. Kroemer et al Annu. Rev. Immunol. 2013. 31:51–72)*
- Low mutation load, biomarkers, individualised?

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Smart Combinations

- Opportunities for synergy through rational design
- TME, heterogeneity & resistance needs to be well studied
- Modulation of the immune microenvironment
- Optimisation of dose, sequence and timing, toxicity
- Personalised approach

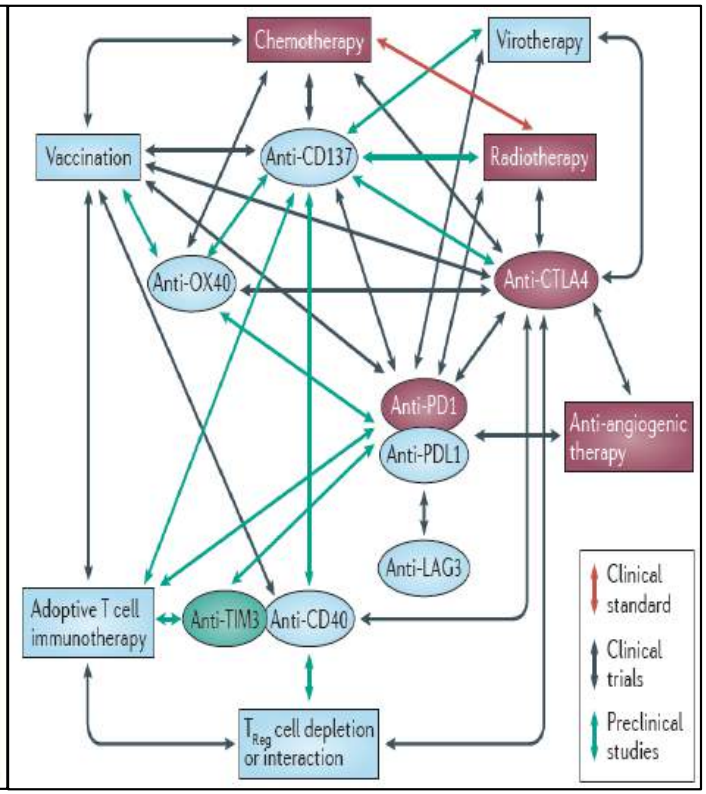
TARGETED AGENTS
+
STANDARD OF CARE
+
IMMUNOTHERAPY

Box 1 | Immunotherapies that are approved or in development

Vaccines
- Dendritic cell-based vaccines
- Autologous granulocyte–macrophage colony-stimulating factor (GM-CSF)-transfected vaccines
- Viral vector vaccines
- mRNA-based vaccines
- Multipeptide-based vaccines
- Locally released virotherapy

Targets of modulatory monoclonal antibodies
- Cytotoxic T lymphocyte-associated antigen 4 (CTLA4)
- Programmed cell death protein 1 (PD1)
- PD1 ligand 1 (PDL1)
- CD137
- OX40
- Lymphocyte activation gene 3 protein (LAG3)
- T cell immunoglobulin and mucin-domain containing 3 (TIM3)
- Glucocorticoid-induced tumour necrosis factor receptor family-related protein (GITR)
- CD27

Adoptive T cell therapy
- Tumour-infiltrating lymphocytes
- Chimeric antigen receptors (CARs)
- CAR-transduced T lymphocytes



Melero et al Nature Review Cancer. 2015 Aug;15(8):457-72

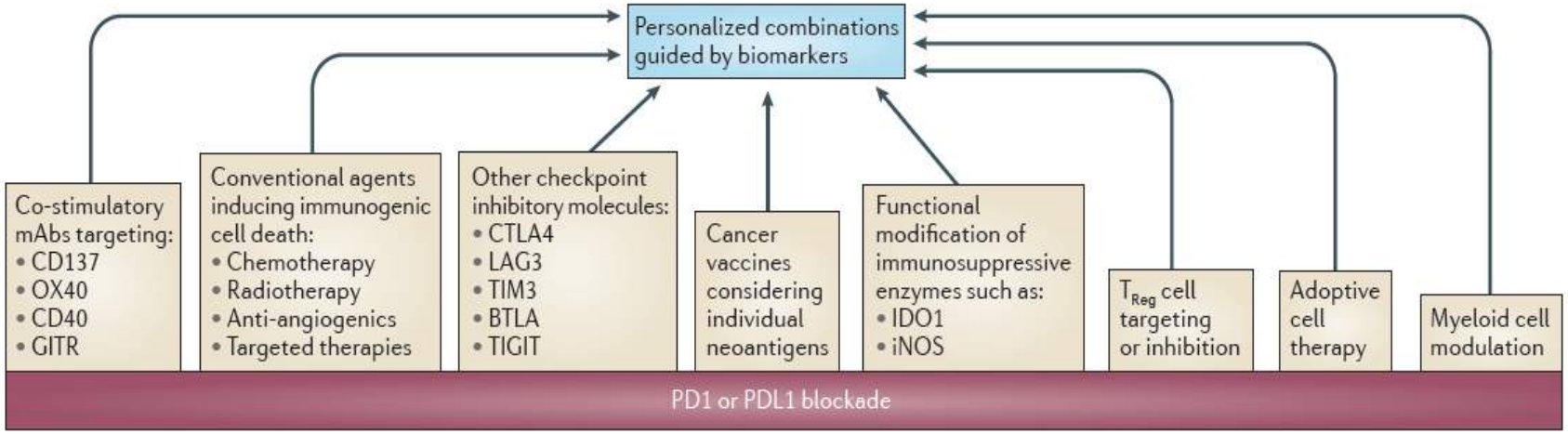
Combinations in the clinic

Breast cancer

- PIII Pembro v Chemo mTNBC
- PIII margetuximab + Herceptin + chemo mHer2-
- PII Pembro +HDACi
- PII Durvalumab + tremelimumab mTNBC or mHER-

Prostate Cancer

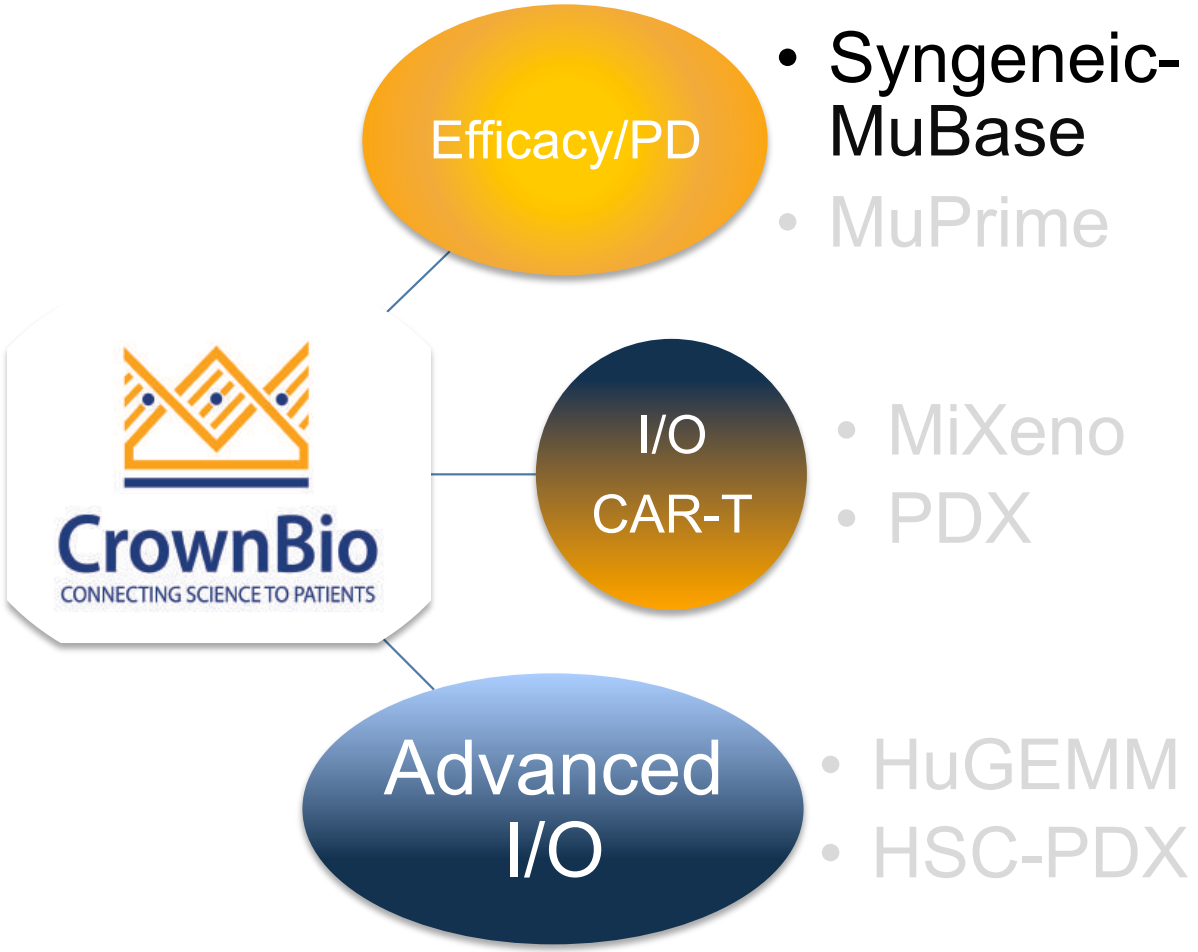
- PIII Ipi + IR mCRPC
- PII Provenge + Indoximod refractory adv PC
- PII T cell therapy targeting NY-ESO-1 + DC vaccine
- DC + androgen ablation



Melero et al Nature Review Cancer. 2015 Aug;15(8):457-72

Murine Models

Global I/O Platform Technologies



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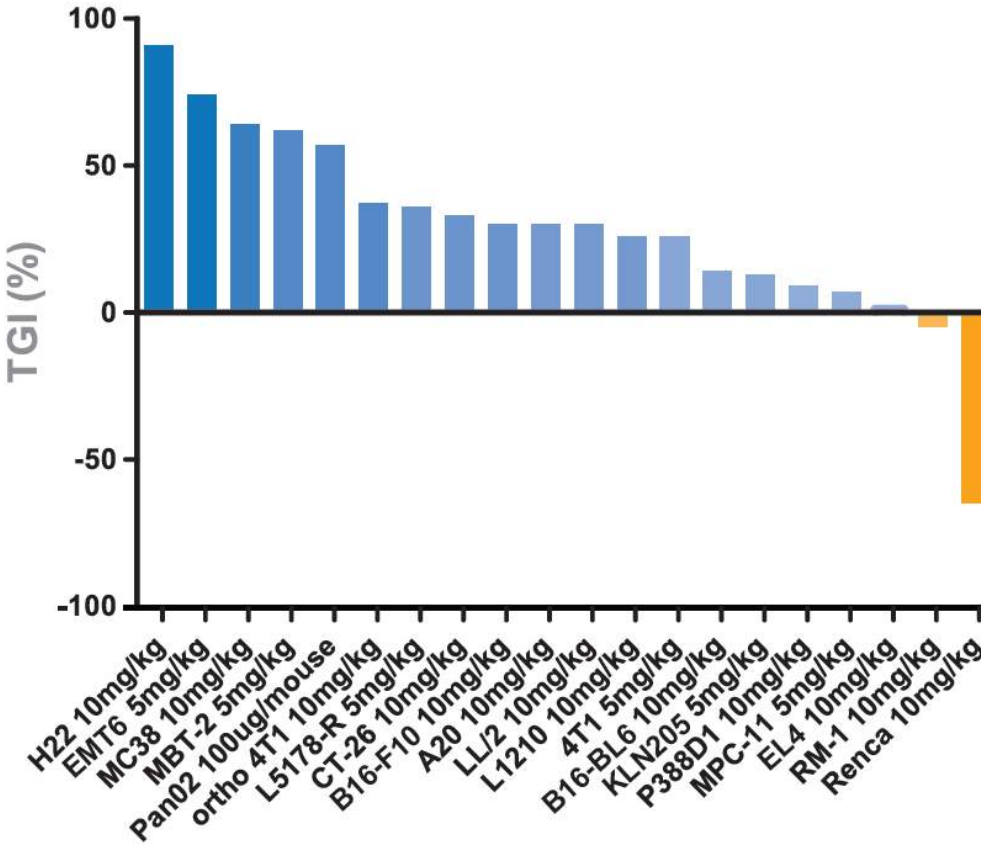
Syngeneic Models: Benchmarking, Characterization & Immunophenotyping

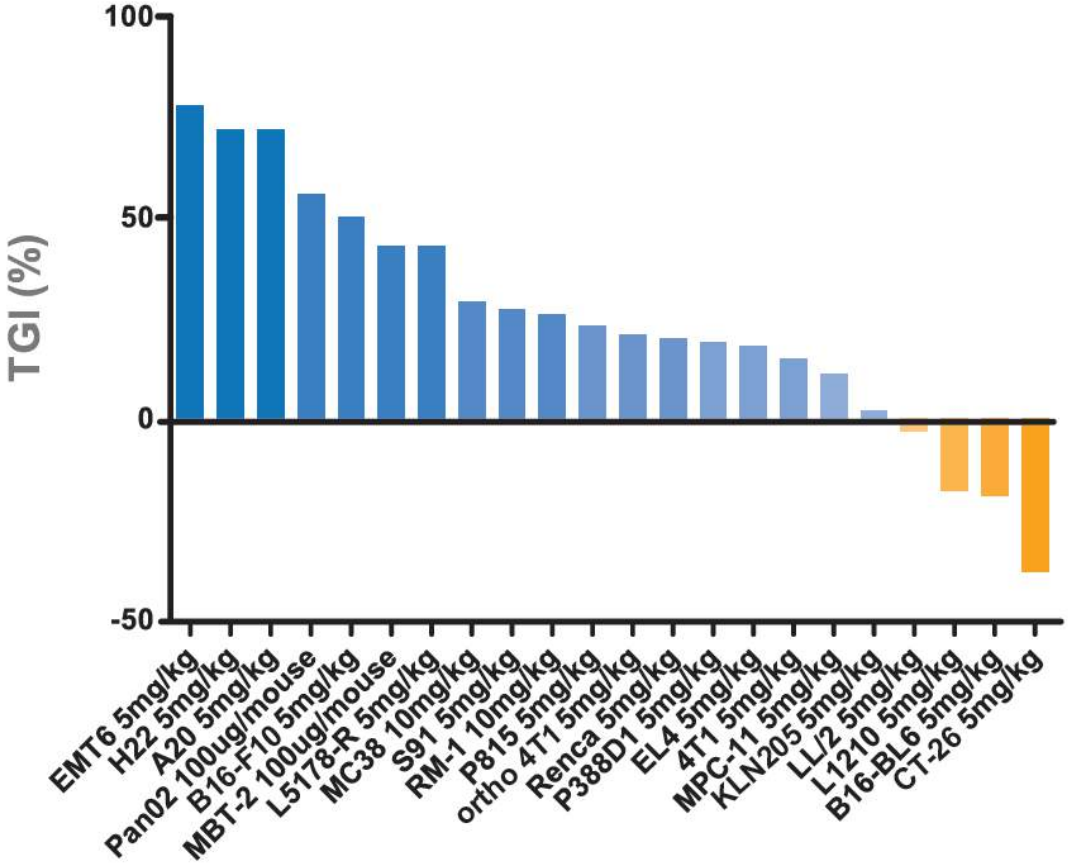
Cancer Type	Cell line	Model type	Mouse Strain	Immune Cell Profiling	RNAseq	Anti-PD-1	Anti-PD-L1	Anti-CTLA-4
Bladder cancer	MBT-2	s.c.	C3H	Yes	Ongoing	Yes	Yes	Yes
Breast cancer	4T1	s.c./ortho/mets	BALB/c	Yes	Yes	Yes	Yes	Yes
	EMT 6	s.c./ortho	BALB/c	Yes	Ongoing	Yes (s.c.)	Yes (s.c.)	Yes (s.c.)
	JC	s.c.	BALB/c	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
Colon cancer	Colon 26	s.c.	BALB/c	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
	CT-26	s.c.	BALB/c	Yes	Yes	Yes	Yes	Yes
	MC38	s.c.	C57BL/6	Yes	Yes	Yes	Yes	Yes
Fibrosarcoma	WEHI-164	s.c.	BALB/c	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
Kidney cancer	Renca	s.c.	BALB/c	Yes	Yes	Yes	Yes	Yes
Leukemia	C1498	s.c.	DBA/2	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
	L1210	s.c.	C57BL/6	Yes	Yes	Yes	Yes	Yes
Liver cancer	H22	s.c./ortho	BALB/c	Yes	Yes	Yes	Yes	Yes
Lung cancer	KLN205	s.c.	DBA/2	Yes	Yes	Yes	Yes	Yes
	LL/2	s.c./mets	C57BL/6	Yes	Yes	Yes	Yes	Ongoing
Lymphoma	A20	s.c.	BALB/c	Yes	Yes	Ongoing	Ongoing	Ongoing
	E.G7-OVA	s.c.	C57BL/6	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
	EL4	s.c.	DBA/2	Yes	Yes	Yes	Yes	Yes
	L5178-R	s.c.	DBA/2	Yes	Ongoing	Ongoing	Ongoing	Ongoing
	P338D1	s.c.	C57BL/6	Yes	Ongoing	Ongoing	Ongoing	Ongoing
Mastocytoma	P815	s.c.	DBA/2	Ongoing	Ongoing	Yes	Yes	Yes
Melanoma	B16-BL6	s.c.	C57BL/6	Yes	Yes	Yes	Yes	Yes
	B16-F10	s.c./ortho/mets	C57BL/6	Yes	Yes	Yes	Yes	Yes
	S91	s.c.	DBA/2	Ongoing	Ongoing	Ongoing	Yes	Ongoing
Myeloma	MPC-11	s.c.	BALB/c	Yes	Ongoing	Ongoing	Ongoing	Ongoing
Neuroblastoma	Neuro-2a	s.c.	A/J	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
Pancreatic cancer	Pan02	s.c.	C57BL/6	Ongoing	Yes	Yes	Yes	Yes
Plasmacytoma	J558	s.c.	BALB/c	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
Prostate cancer	RM-1	s.c.	C57BL/6	Yes	Ongoing	Yes	Yes	Yes

Anti-PD-1/PD-L1 Benchmarking

aPD-1 (RMP1-14)

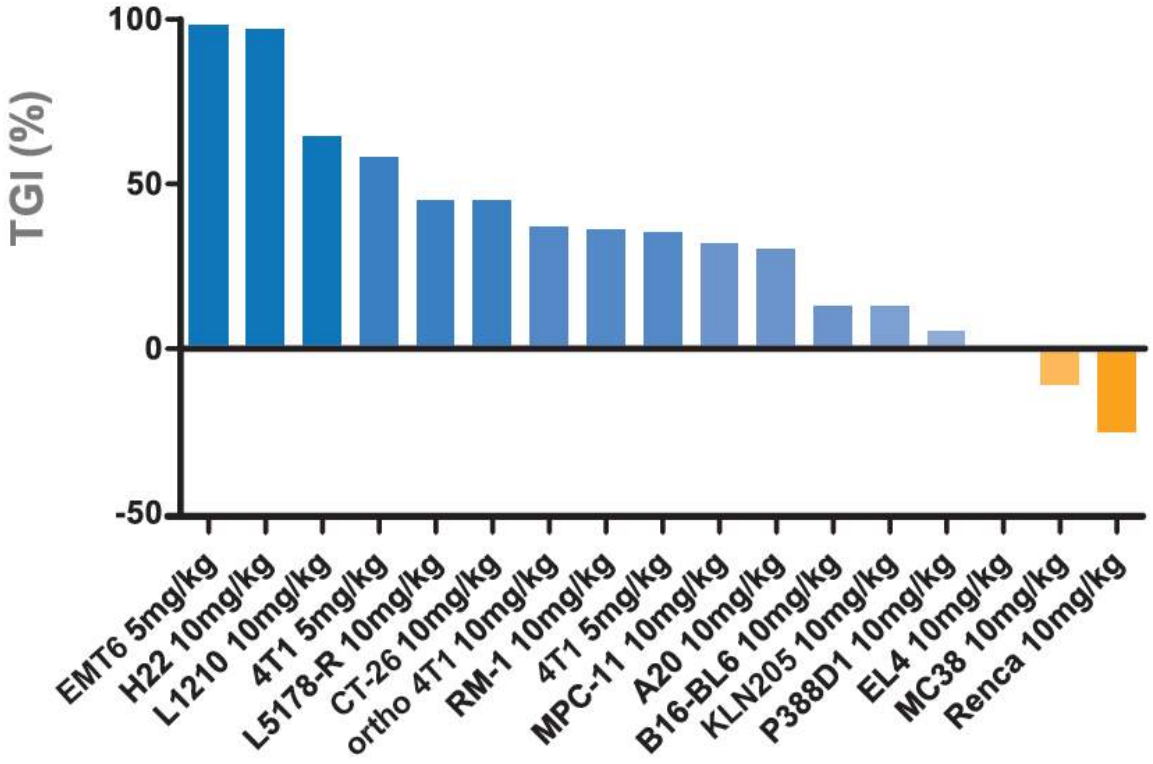


aPD-L1 (10F.9G2)

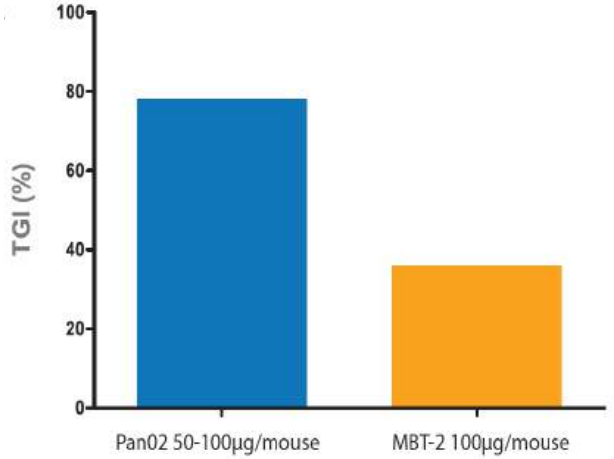


Anti-CTLA-4 Benchmarking

- Efficacy evaluation of aCTLA-4 (9D9) in syngeneic models



- Efficacy evaluation of aCTLA-4 (9H10) in syngeneic models



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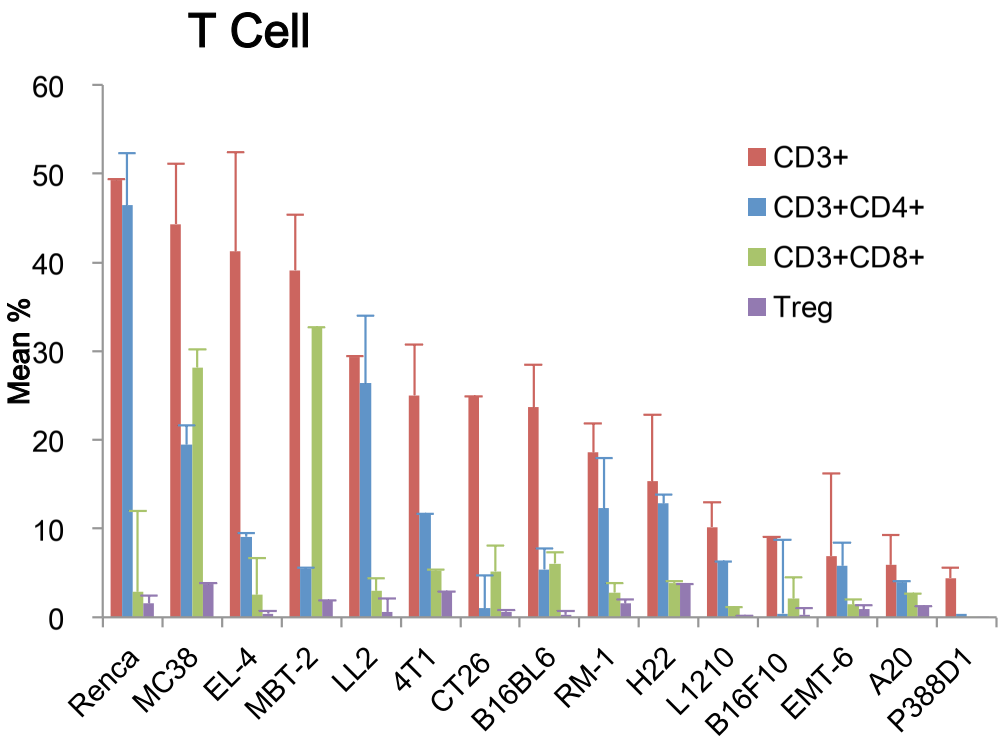
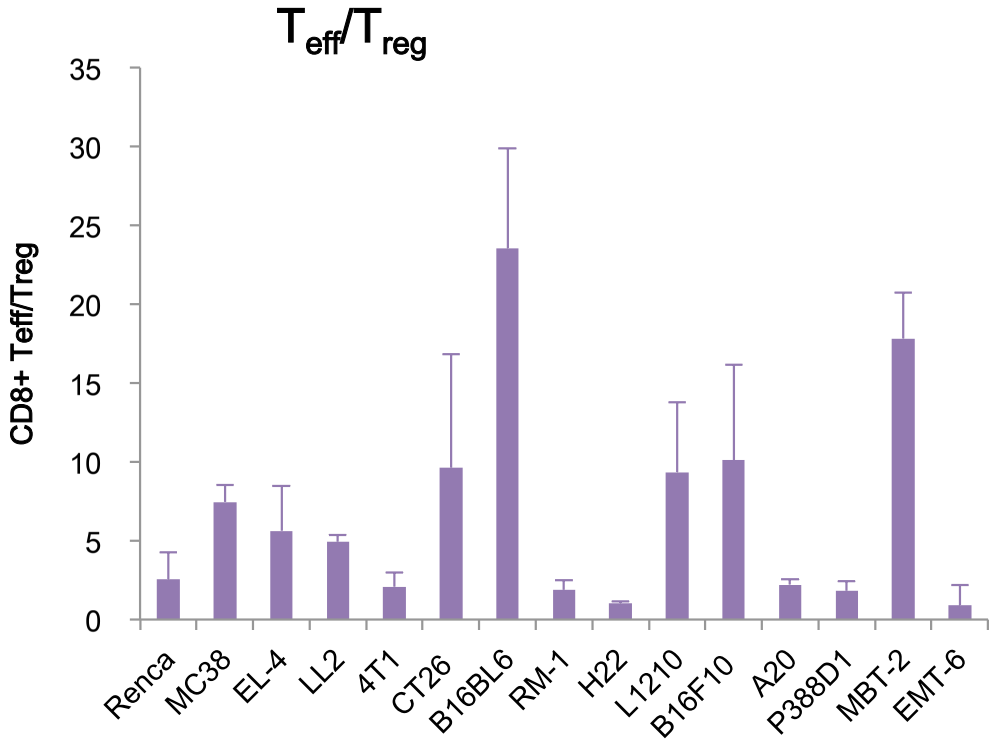
Base Line Immuno phenotyping





Depletion of CD8+ promotes MC38 tumour growth

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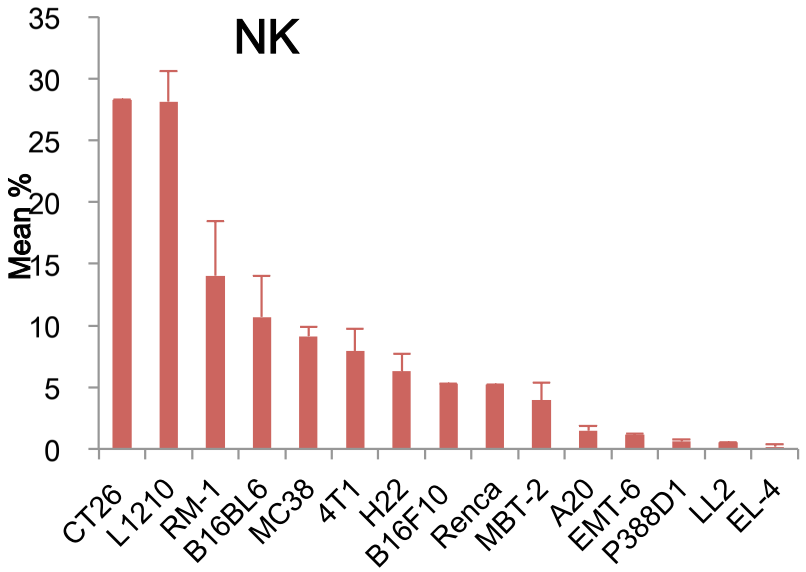
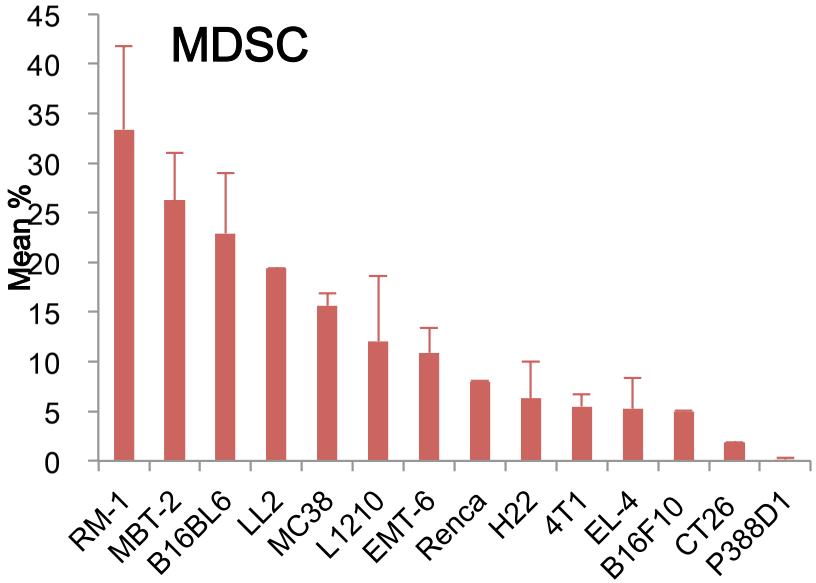
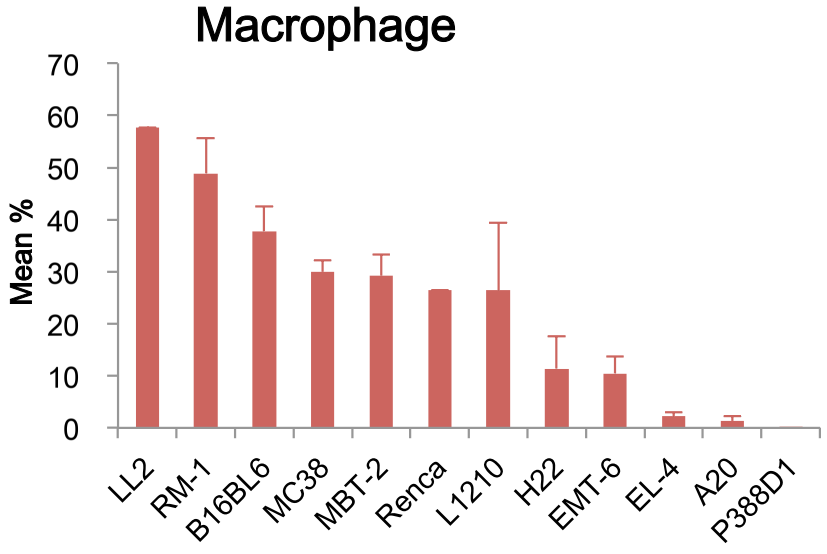
Base Line Immuno phenotyping

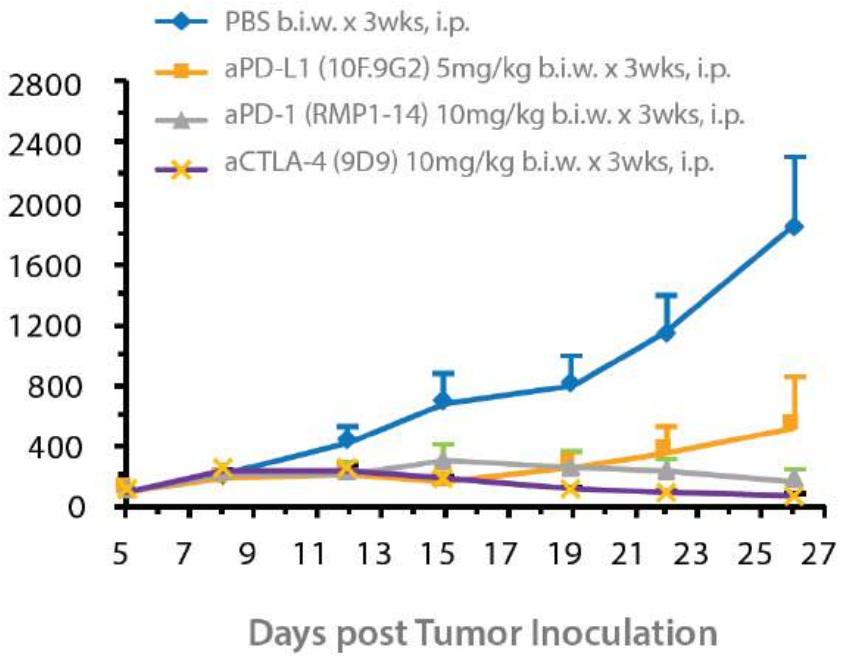




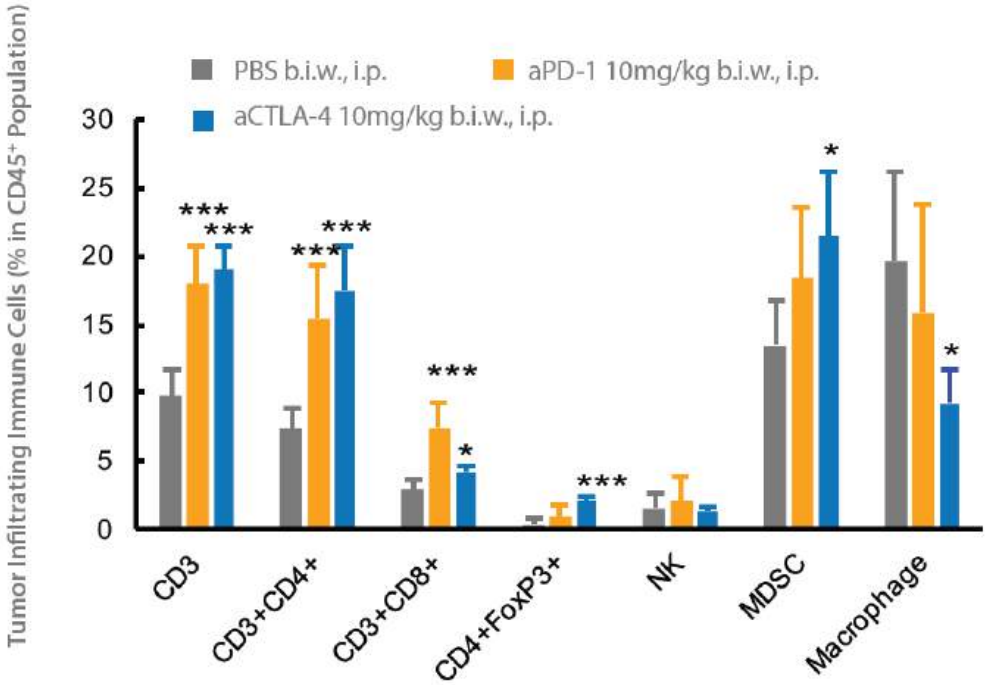


H22 Checkpoint Antibodies Response & immunophenotyping

Highly sensitive to anti-CTLA-4, PD-1, and PD-L1 therapy

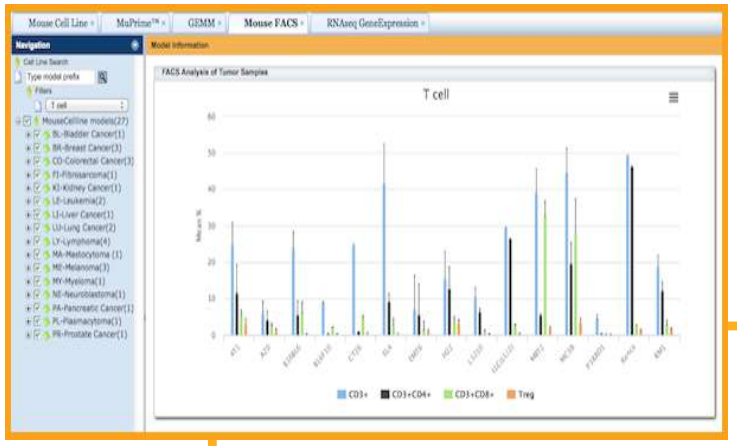


H22 tumor on Day 21: 2 days post 5th dose



Treatment	Tumor Size (mm^3) on Day 26	T/C Value (%) on Day 26	p value
PBS	1843 ±466	--	--
aPD-L1 (10F.9G2) 5mg/kg	520 ± 340	28	0.051
aPD-1 (RMP1-14) 10mg/kg	165 ±73	9	0.007
aCTLA-4 (9D9) 10mg/kg	56 ±9	3	0.005

Oncology Knowledge Databases



OncoExpress

MuBase

- **The world's first commercial database dedicated to mouse cancer models**
- Free online access to search **Syngeneics, GEMM, MDX (MuPrime), HuGEMM™**
- Make an informed decision by searching models based on gene expression, mutational status, gene fusions, etc. across our unique collection of mouse cancer models
- Wide variety of data compiled including model background, mouse strain, histopathology, genomic profiling (RNAseq), SoC, and immuno-profiling (infiltrate immune cells, cytokine profile, response to checkpoint inhibitors, etc.)

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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	ENSMUST00000020329	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	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	-

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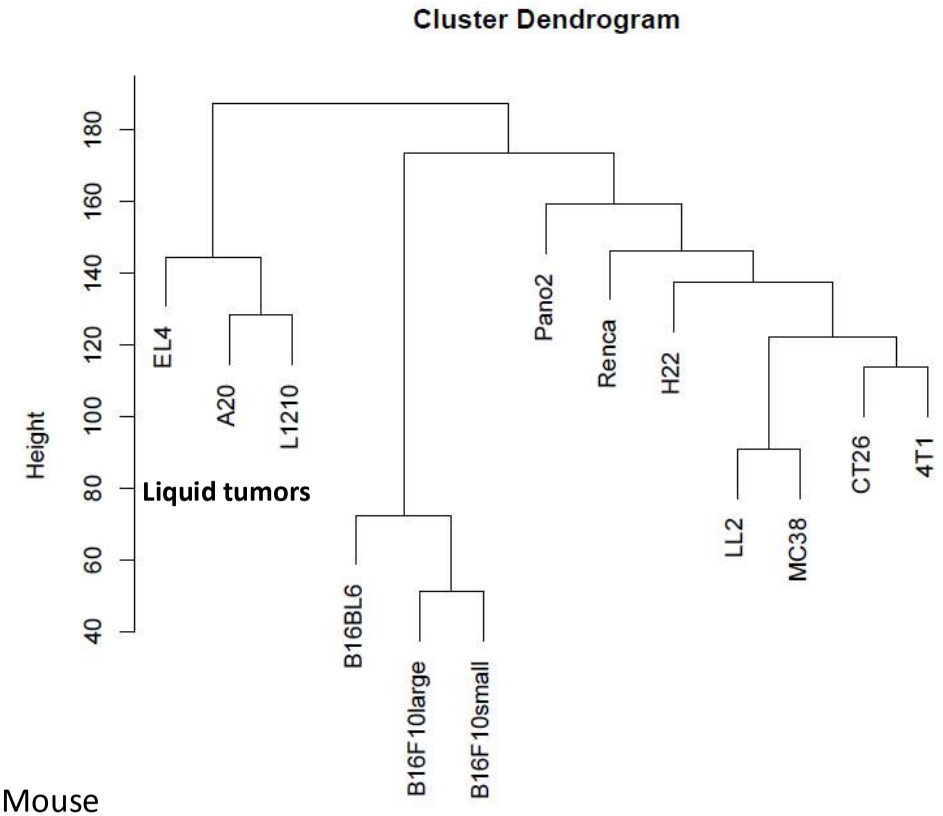
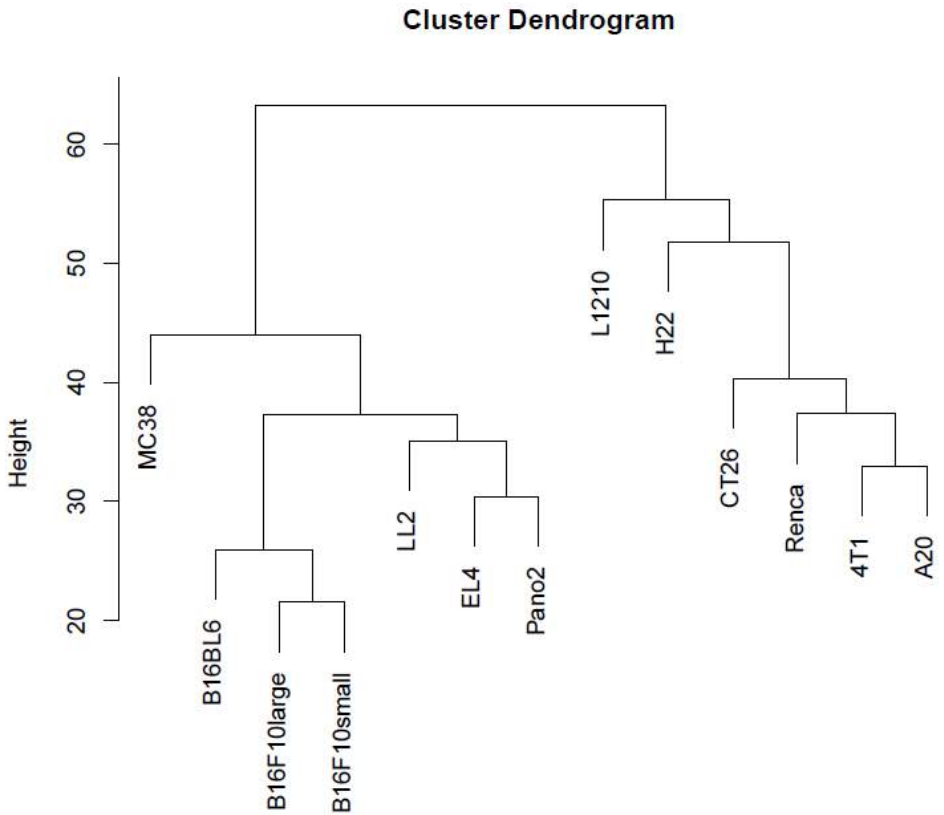
Whole Genome Gene Fusion Analysis: 4T1 Model

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
Ctla2b	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
Kntc1	chr5	+	1.24E+08	E	Snrnp35	chr5	+	1.25E+08	E	3	11	INTRACHR-SS-OGO-10GAP	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

Tumor Models Clustering by Gene Expression and Mutation



By Expression



By Mutation

Zhang et al, AACR New Horizons in cancer research 2015, Shanghia, Poster 246

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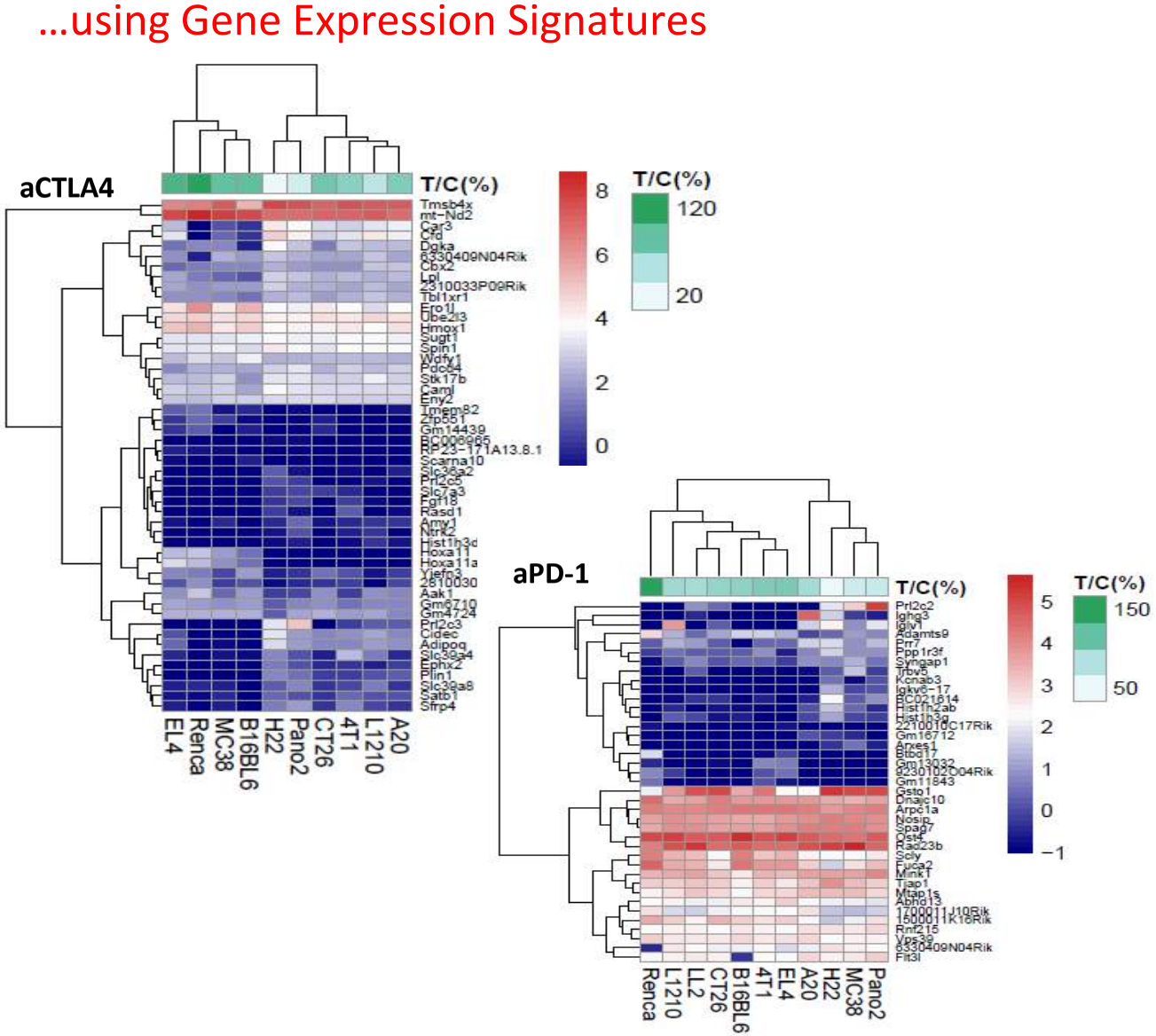
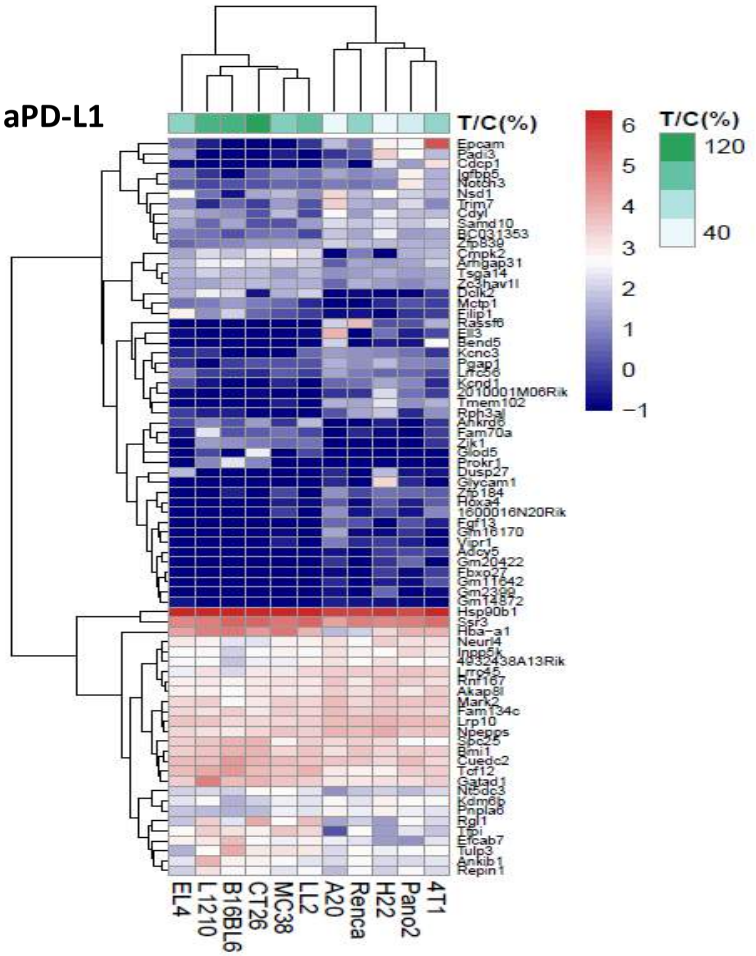
Efficacy Prediction of Immune Checkpoint Inhibition (I)

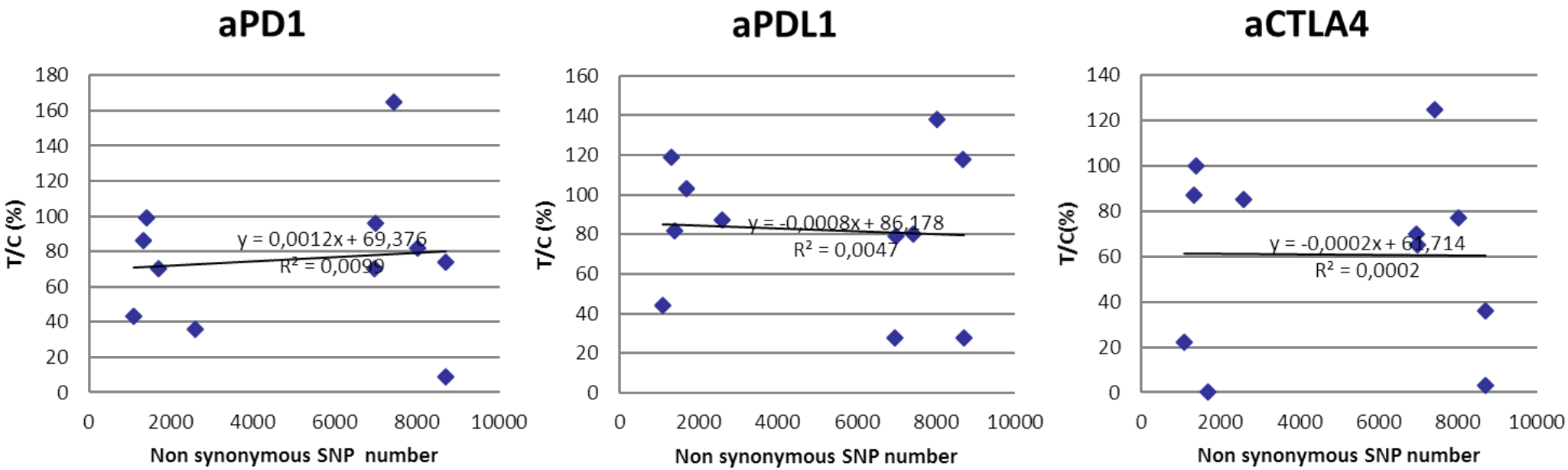
…using Gene Expression Signatures





Efficacy Prediction of Immune Checkpoint Inhibition (II)

…based on mutational load
…DNA repair gene mutations



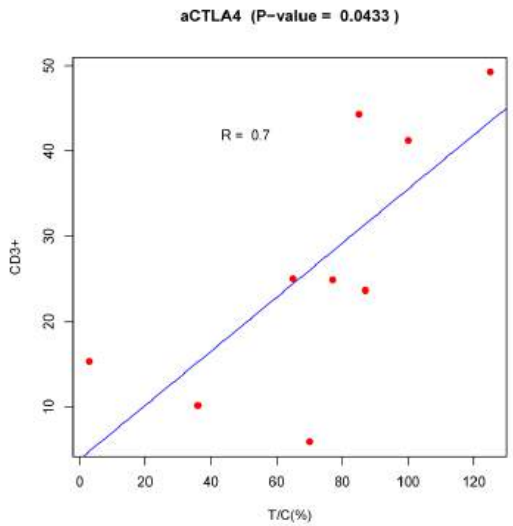
No correlation was found…

Zhang et al, AACR New Horizons in cancer research 2015, Shanghia, Poster 246

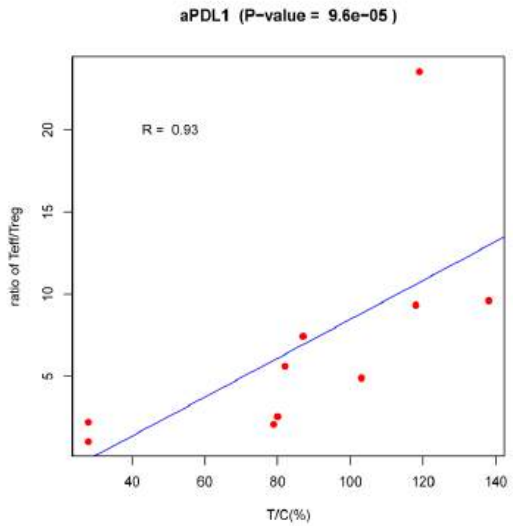
Efficacy Prediction of Immune Checkpoint Inhibition (III)

…based on base line Immune Cells data

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	-0.200608829	0.614067751	0.182371663
Treg	0.743540564	-0.133333333	0.326657258	-0.34650616	0.069155844	-0.595747433
ratio of Teff/Treg	0.3125	0.383333333	0.626123419	0.176292608	9.60E-05	0.930095482
NK	0.46299052	-0.283333333	0.789234533	-0.097264887	0.072785937	0.589668377
MDSC	0.708068783	0.15	0.72525014	-0.127660164	0.22755151	0.419454825
Macrophage	0.555952381	0.285714286	0.977546655	0.011976263	0.055884726	0.694623232



Correlation trend was found…



- PD, model selection, rational combination approach but biomarker?

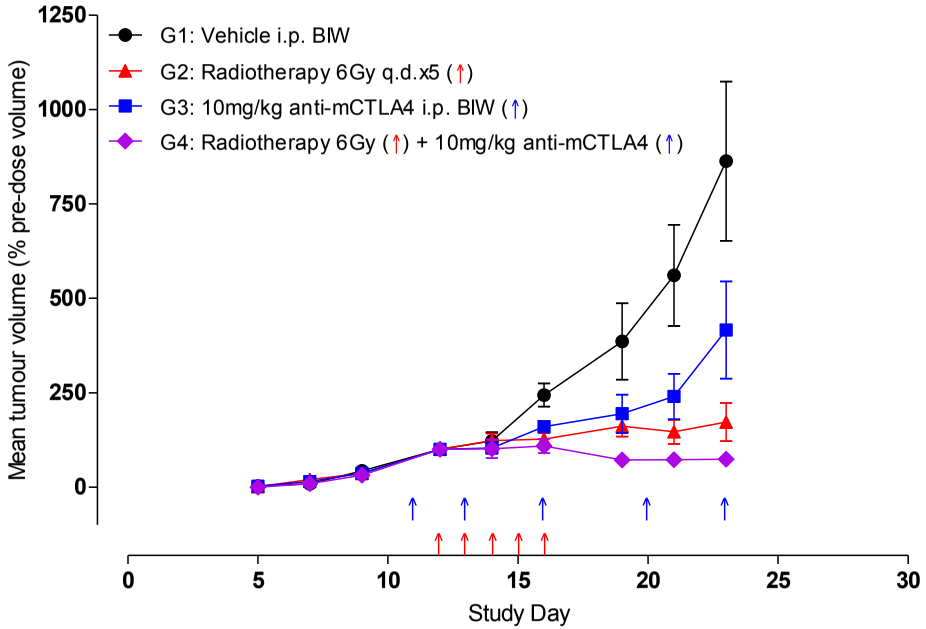
Zhang et al, AACR New Horizons in cancer research 2015, Shanghia, Poster 246

Combination with anti-mCTLA-4 (CT26)

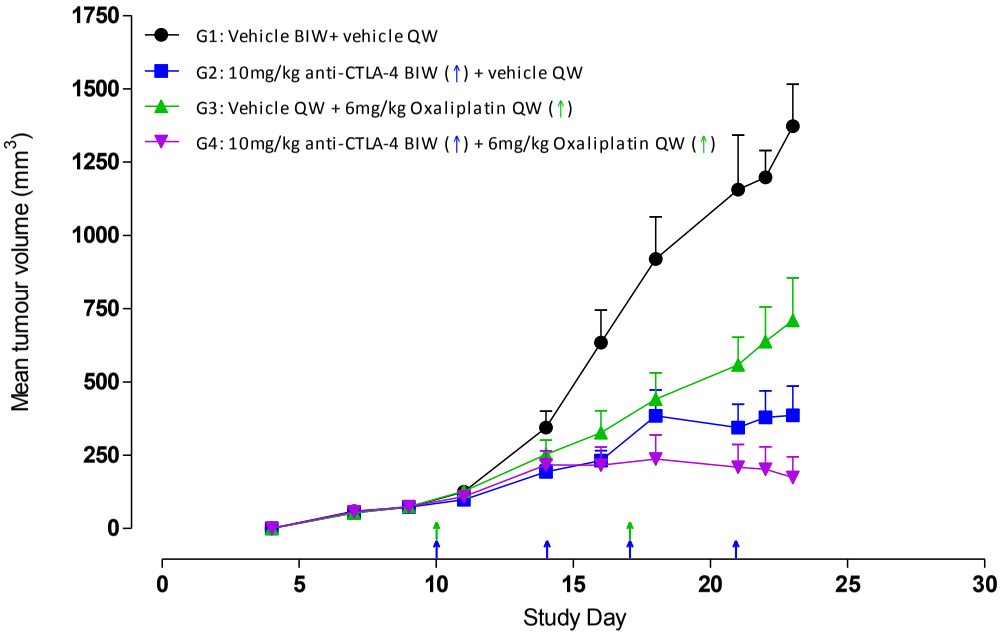
Induction of ICD:

Combination immunotherapy (anti-mCTLA-4) and radiotherapy (RT; hypofractionated IGMI) or Oxaliplatin was assessed alone, and in combination in BALB/c mice bearing subcutaneous CT26 allografts.

CT26 *in vivo* response to combination dosing of anti-mCTLA-4 + RT



CT26 *in vivo* response to combination dosing of anti-mCTLA-4 + Oxaliplatin

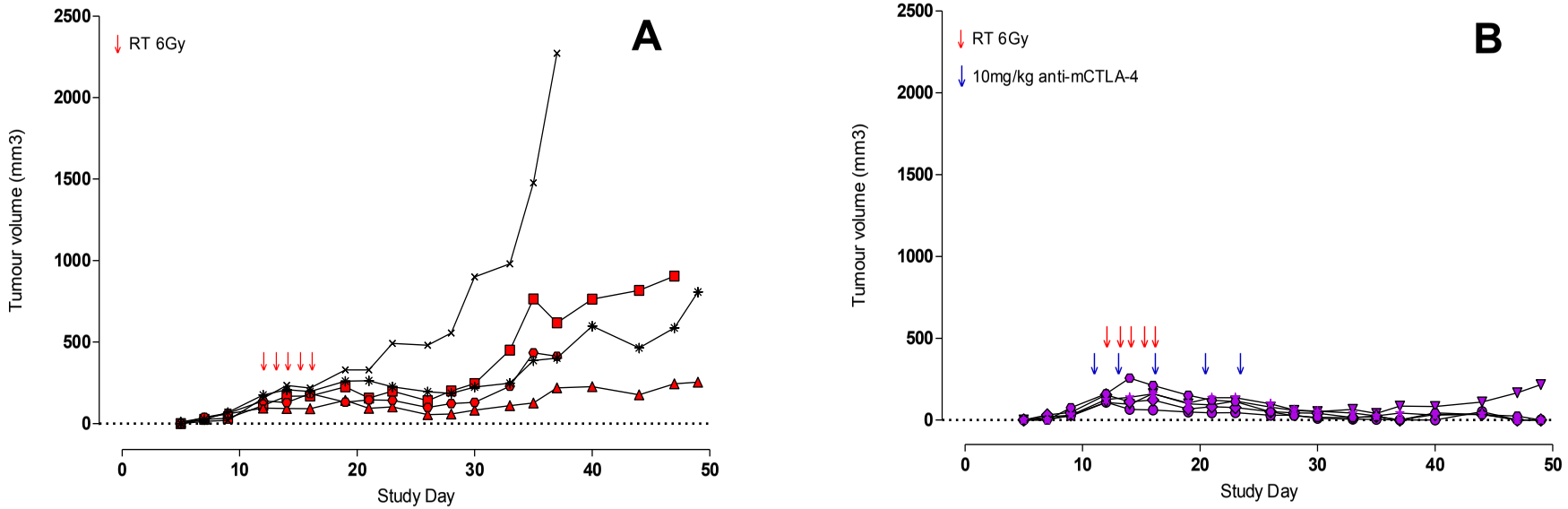


McKenzie et al AACR 2016, New Orleans

- Anti-mCTLA-4 and ICD monotherapy results in a statistically significant reduction in tumour growth ($p<0.05$) compared with vehicle treatment.
- Anti-mCTLA-4 therapy in combination with radiotherapy resulted in statistically significant additive reduction in tumour growth ($p<0.05$ Two-way ANOVA) versus anti-mCTLA-4 and RT monotherapies.

Long term combination effect (CT26)

CT26 *in vivo* outgrowth following A. RT treatment and B. anti-mCTLA-4 + RT



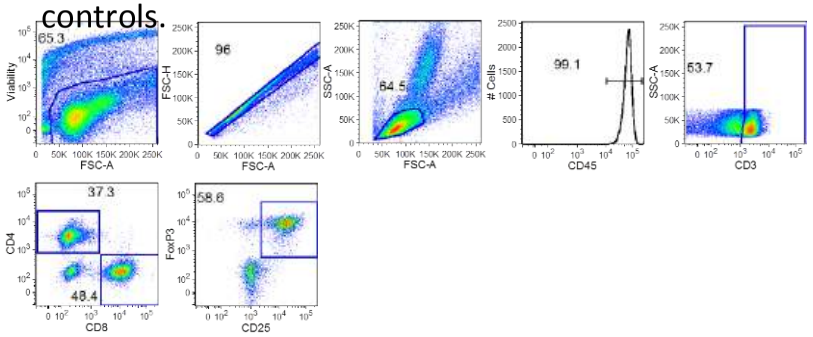
McKenzie et al AACR 2016, New Orleans

- Anti-mCTLA-4 and RT combination treated CT26 tumours, regression continued following cessation of treatment with a delay in tumour growth observed in comparison to RT alone.
- Markers of ICD (Calreticulin & HMGB1) TBD

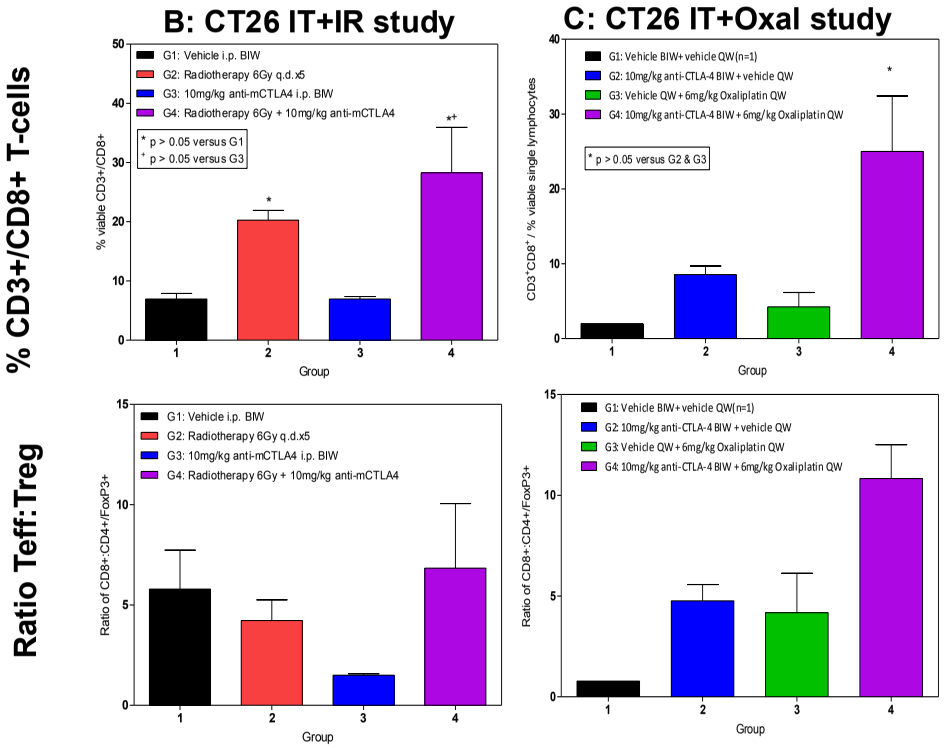
Increased CD8+ T cells (CT26)

Cytotoxic T-cells (Teff; CD3+/CD8+) and Treg (CD4+/CD25+/FoxP3+) tumour infiltration was assessed at day 23 in by FACs in CT26 tumours following treatment with immunotherapy (anti-mCTLA-4) and ICD alone and in combination

Representative flow cytometry plots: A-D.: Selection of single, viable lymphocytes. E.-F. CD4 vs CD8 T cells. F.-G. CD4+CD25+FoxP3+ T regulatory cells. Gating strategy was performed based on FMO controls.



- Significant increase in CD8+ T-cells for both ICD and IT+ICD compared with vehicle control and versus IT alone for the IT+ICD group.
- Trend in increasing CD8+ T-cells for the IT+RT versus RT alone (which may be driving the additive efficacy seen in this treatment group) and significance for IT+Oxal.
- Treat larger sized tumours TBD



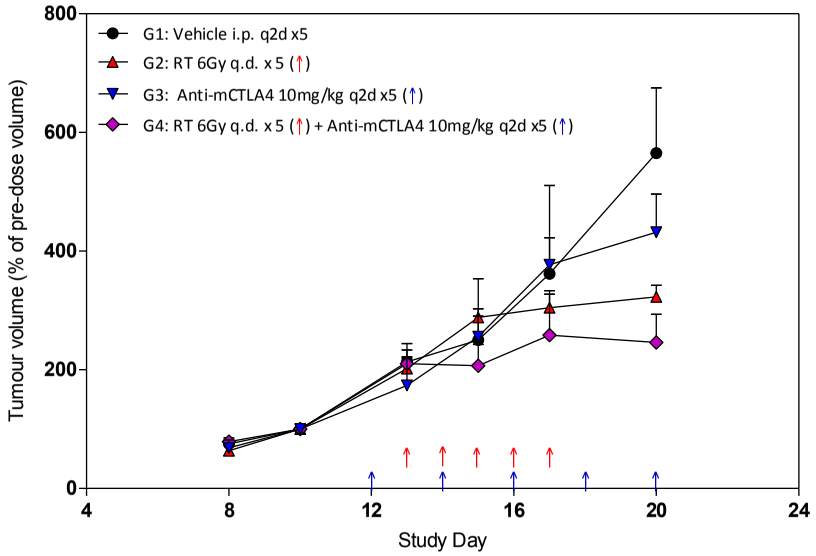
Flow cytometry analysis of subcutaneous tumour

McKenzie et al AACR 2016, New Orleans

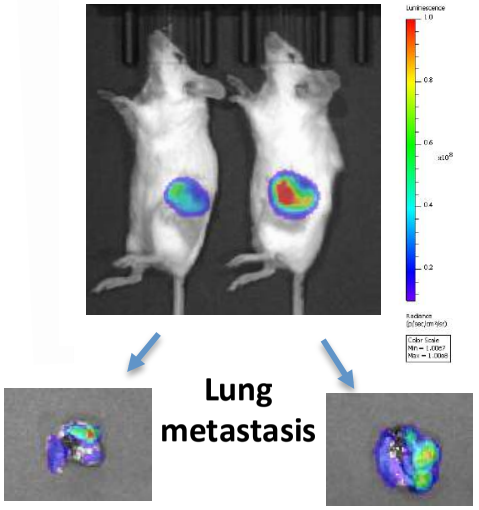
4T1 Mu-lux orthotopic/metastatic breast cancer syngeneic model

Bioluminescent breast carcinoma cell line 4T1-lux, metastasizes from the MFP > lung, lymph, liver and brain; and from s.c. site to the lung. Metastasis can be assessed by terminal *ex vivo* imaging.
The effect of monotherapy/combination therapy with 10mg/kg anti-mCTLA-4 i.p. q2d. (↑) and radiotherapy 6Gy qdx5 (↑) on tumour growth is detailed below.

In vivo response to combination dosing of anti-mCTLA-4 + RT

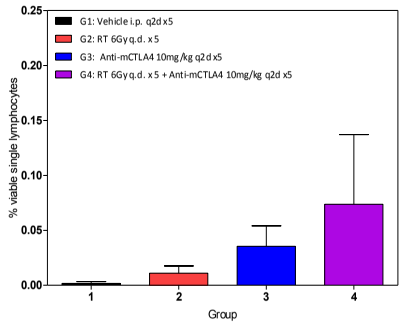


Ex vivo BLI of metastatic lung lesions from s.c. tumour growth

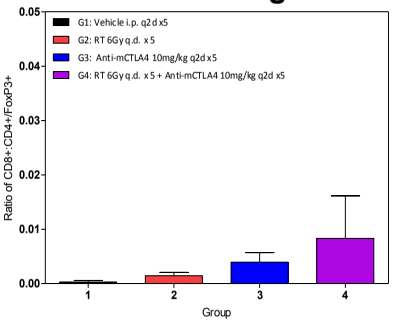


Flow cytometry analysis of 4T1 metastatic lung tumour

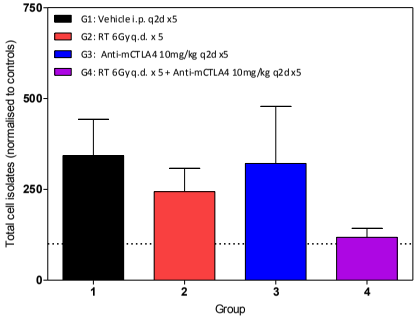
% CD3+/CD8+ T-cells



Ratio Teff:Treg



Whole lung total cell isolates

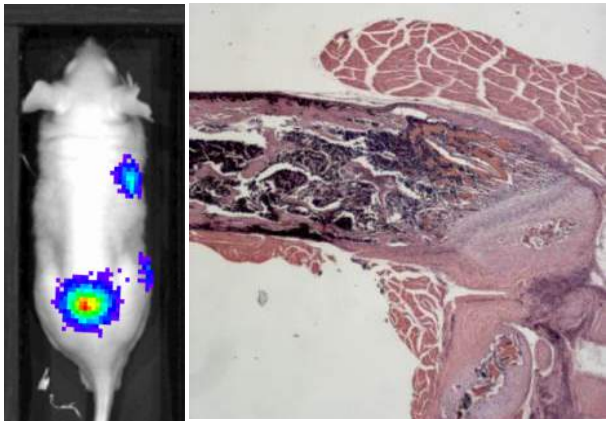


Mu-lux models under development

Ongoing

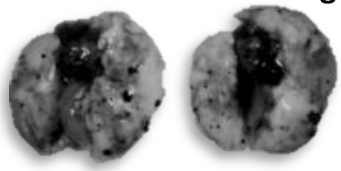
- Establishment at sc site ongoing for all syngeneic lines
- Transduction of remaining panel ongoing
- Bioluminescent syngeneic models, sc versus orthotopic ongoing for Pan02, H22, RM-1
- Metastasis assessment at end stage
- Experimental/seeding metastatic model development e.g. B16-F10
- Immune cell infiltration quantification & baseline data with FACs, Luminex, IHC
- Validation with immuno- therapeutics and combinations

Bioluminescent B16-F10-lux mouse melanoma cells metastasise to bone following intracardiac inoculation. Metastasis can be assessed by terminal *ex vivo* imaging.



In-life BLI | Histological confirmation of melanoma metastasis

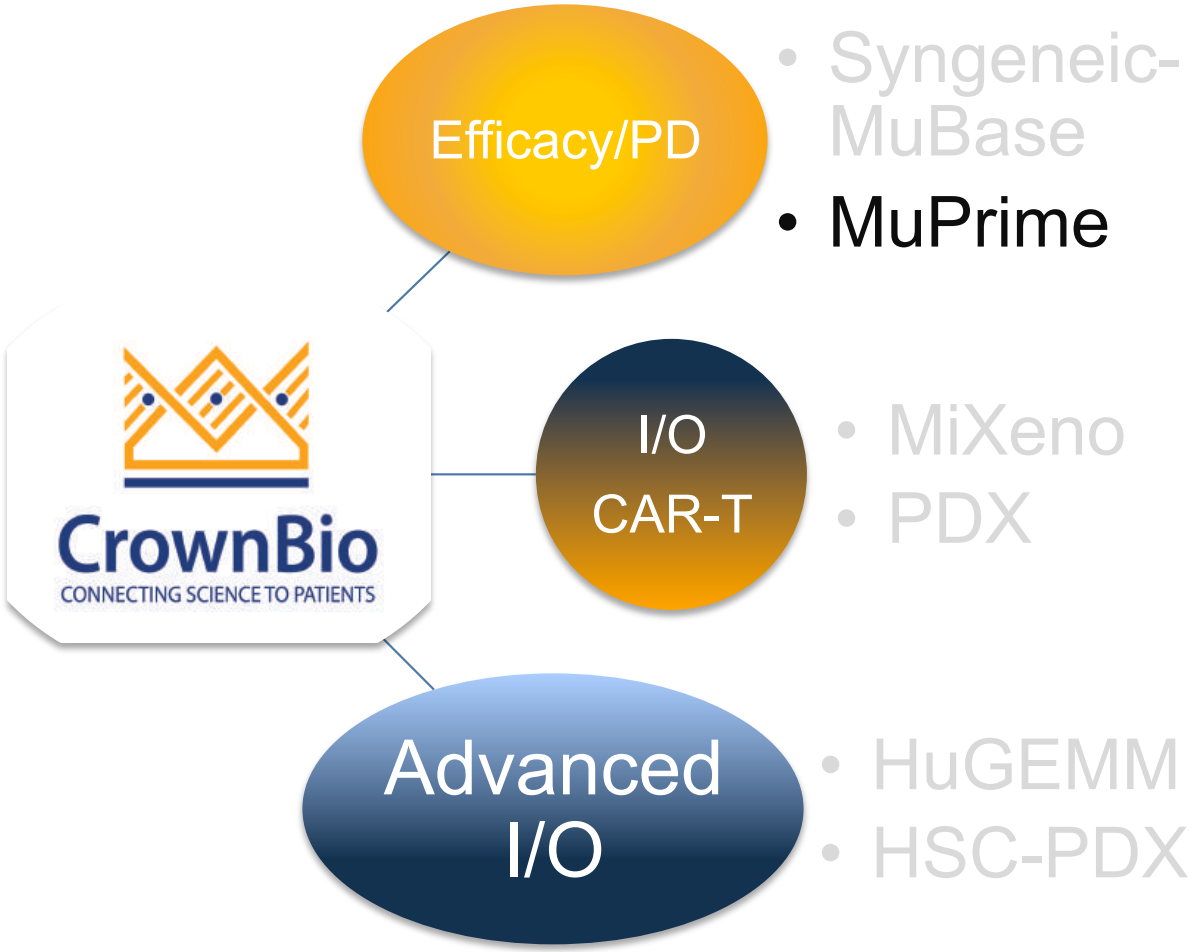
B16-F10-lux cells injected i.v. metastasise to the lungs



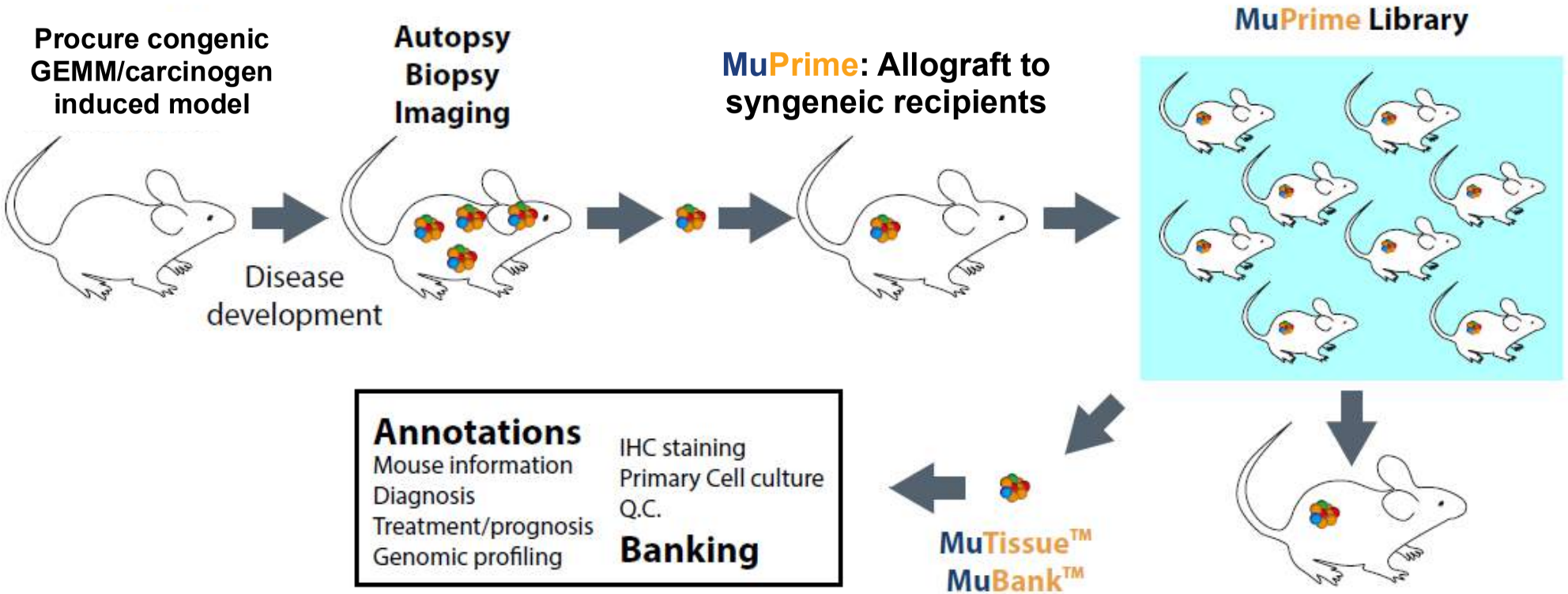
Quantified by direct evaluation of macroscopic tumour deposits and *ex vivo* bioluminescent imaging.

New Murine Models

Global I/O Platform Technologies



Overview of MuPrime Technology



GEMM allografts for advanced I/O studies

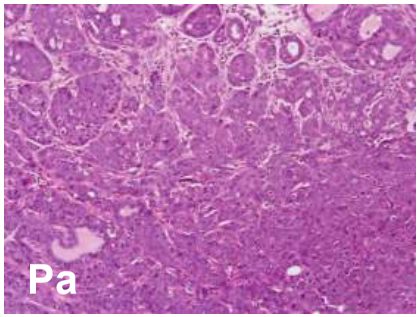
CrownBio
CONNECTING SCIENCE TO PATIENTS

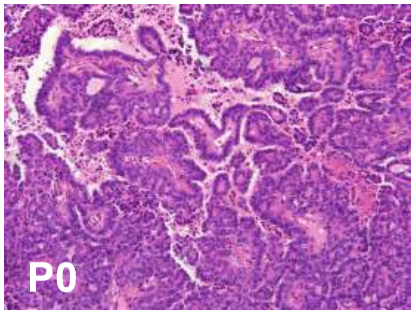
Summary of MuPrime Models

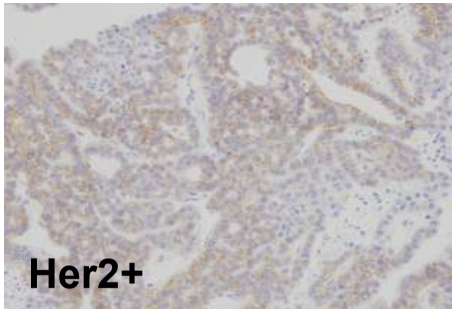
MuPrime ID	Cancer Type	Mutations/Carcinogen	Strain Background	Tumor Progression	MuPrime Setup	MuPrime Validation	Efficacy Ready?
Traditional GEMM-Derived MuPrime							
mPR6003	Prostate	TRAMP (Pbsn-SV40T TG)	C57BL/6			Ongoing	Q3, 2016
mBR6004	Breast	MMTV-PyVT TG	FVB/N				Ready
mSK6005	Skin squamous cell carcinoma	$Apc^{Min/+}$	C57BL/6				Ready
mLY6043	B cell lymphoma	IgH-Myc TG (Eμ Myc)	C57BL/6				Ready
mSA9003	Sarcoma	$P53^{-/-}$	C57BL/6				Ready
mLY6041	B cell lymphoma	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
mLU6042, mLU6044, mLU6045	Lung	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
mCR6046	Colon	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
mLI6047	Liver	KRAS (G12D); $P53^{-/-}$	C57BL/6				Ready
	Pancreatic	KRAS (G12D); $P53^{-/-}$	C57BL/6	Ongoing			Q2, 2016
	Prostate	KRAS (G12D); $PTEN^{Flox/Flox}$	C57BL/6	Ongoing			Q4, 2016
	Ovarian	KRAS (G12D); $PTEN^{Flox/Flox}$	C57BL/6	Ongoing			Q3, 2016
	Bladder	KRAS (G12D); $PTEN^{Flox/Flox}$	C57BL/6	Ongoing			Q3, 2016
	Lung	KRAS (G12D); $PTEN^{Flox/Flox}$	C57BL/6		Ongoing		Q2, 2016
	Glioblastoma	KRAS (G12D); $PTEN^{Flox/Flox}$	C57BL/6	Ongoing			Q3, 2016
	Bladder	$PTEN^{Flox/Flox}$; $P53^{-/-}$	C57BL/6	Ongoing			Q2, 2016
	Prostate	$PTEN^{Flox/Flox}$; $P53^{-/-}$	C57BL/6	Ongoing			Q4, 2016
	Pancreatic	KRAS (G12D); $P16^{-/-}$	C57BL/6	Ongoing			Q3, 2016
	Lung	KRAS (G12D); $P16^{-/-}$	C57BL/6	Ongoing			Q3, 2016

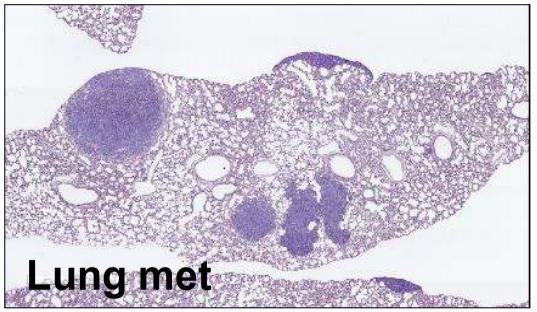
MuPrime mBR6004

Parental GEMM strain	Strain background	Pathology diagnosis
FVB/N-Tg（MMTV-PyVT)634Mul/Jnju	FVB/N	Breast cancer

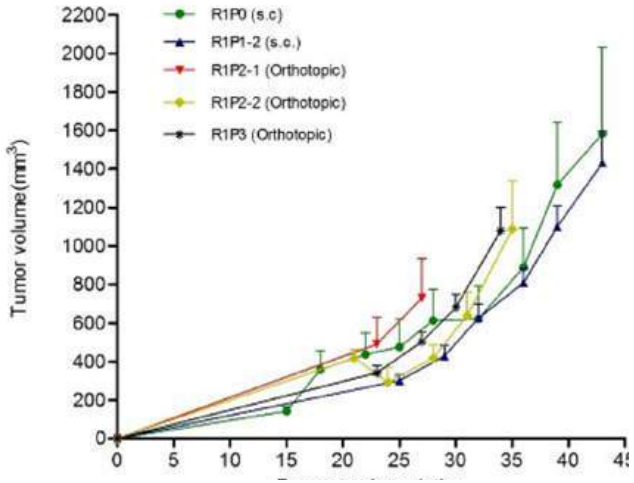


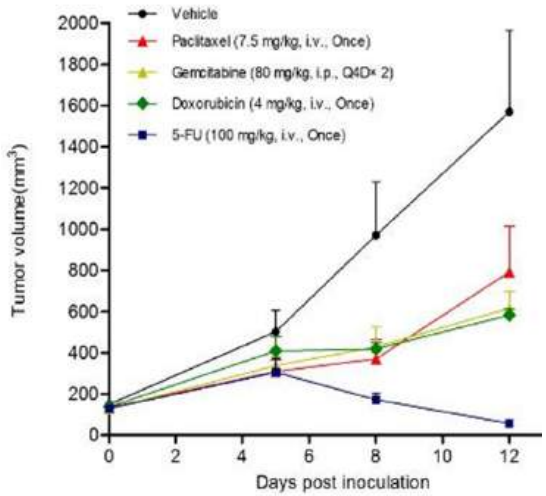






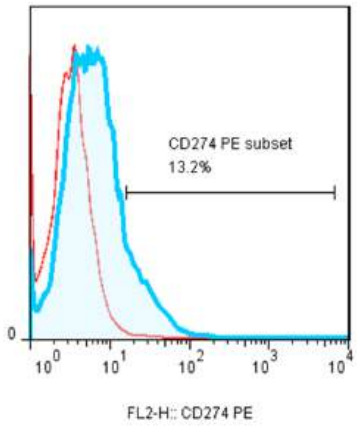
EGFR	FS-insertion 1431G->GA
ERBB2	A130T
MET	I851M
KRAS	WT
BRAF	WT
AKT1	WT
PIK3CA	WT
CTNNB1	WT
PTEN	WT



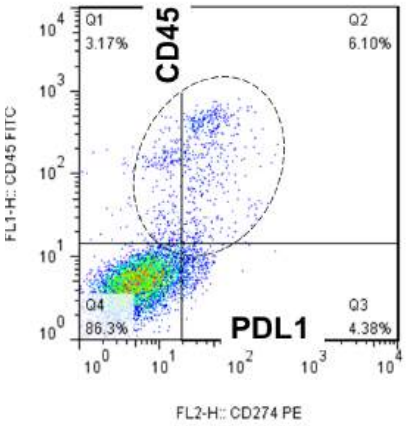


mBR6004 Immune Profiling and Response to Checkpoint Inhibitors

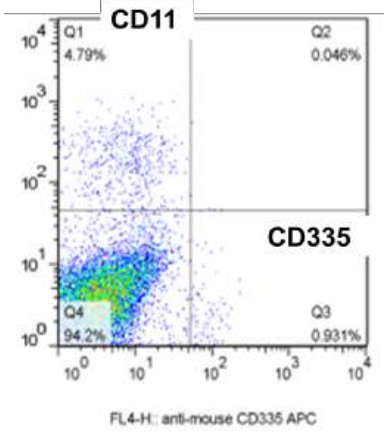
PD-L1 Expression



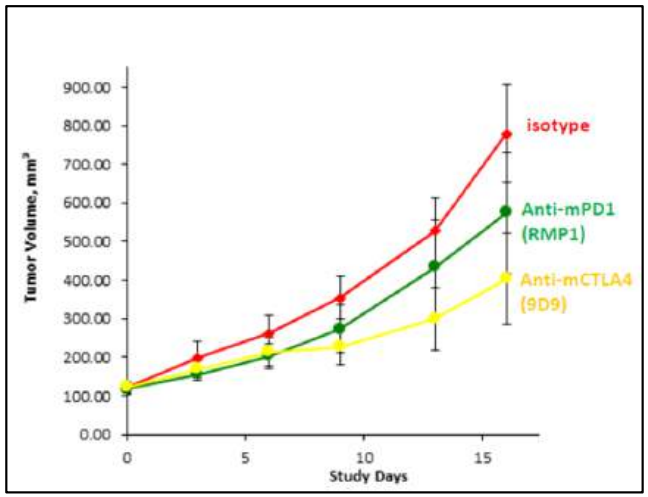
Baseline Infiltrating Immune Cells



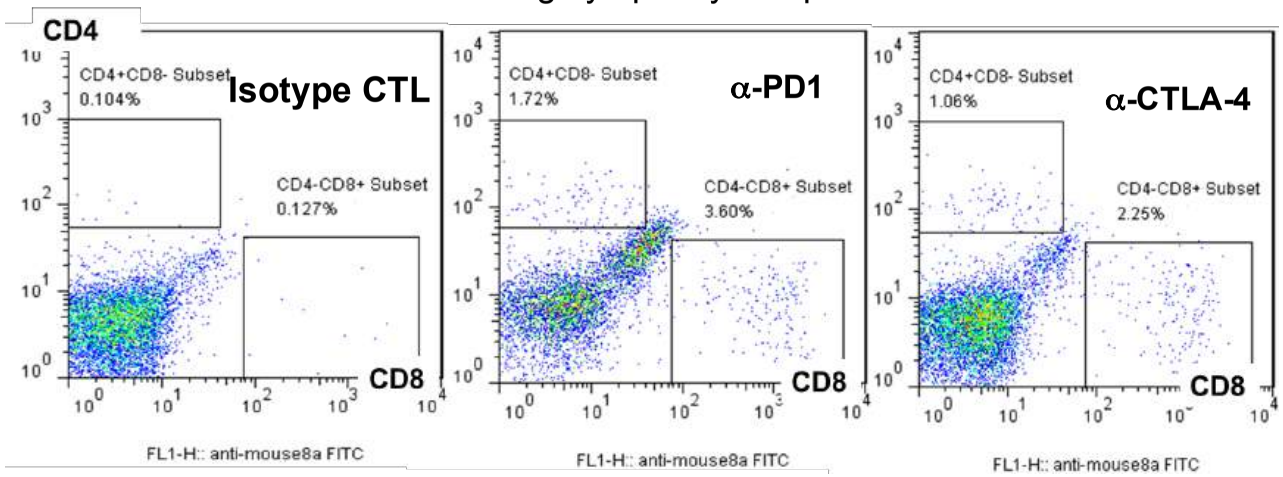
Baseline Infiltrating NK/Neutrophils



mBR6004: Partial Response to I/O Therapies



Tumor-Infiltrating Lymphocytes upon Treatment

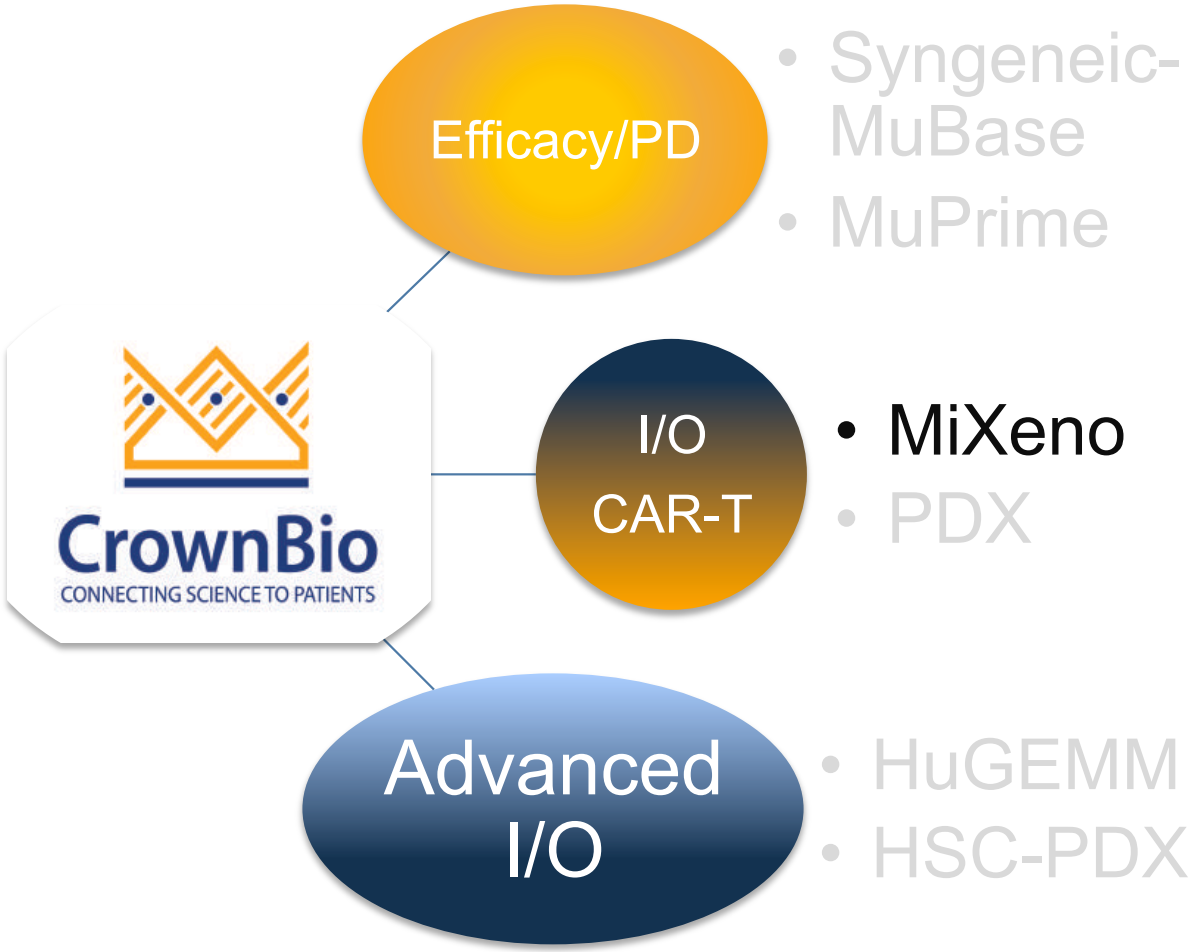


Efficacy & TIL Summary

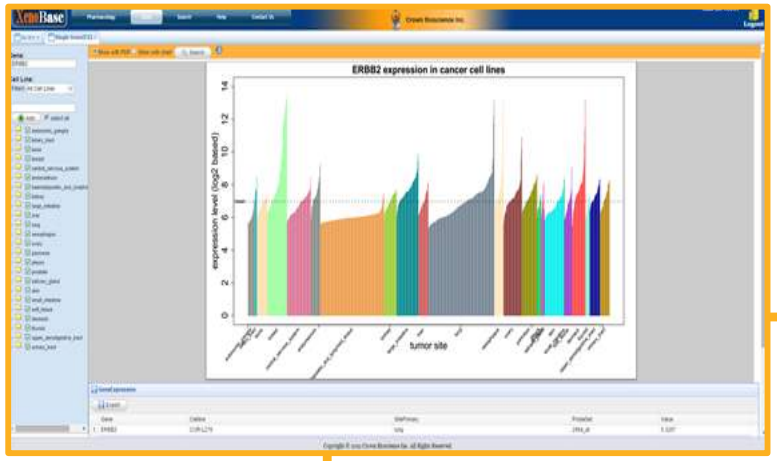
	TV (mm^3)	CD4%	CD8%
Isotype CTL	829	0.104	0.127
α-PD1	438	1.72	3.60
α-CTLA-4	345	1.06	2.25

Humanised Systems

Global I/O Platform Technologies



Oncology Knowledge Databases

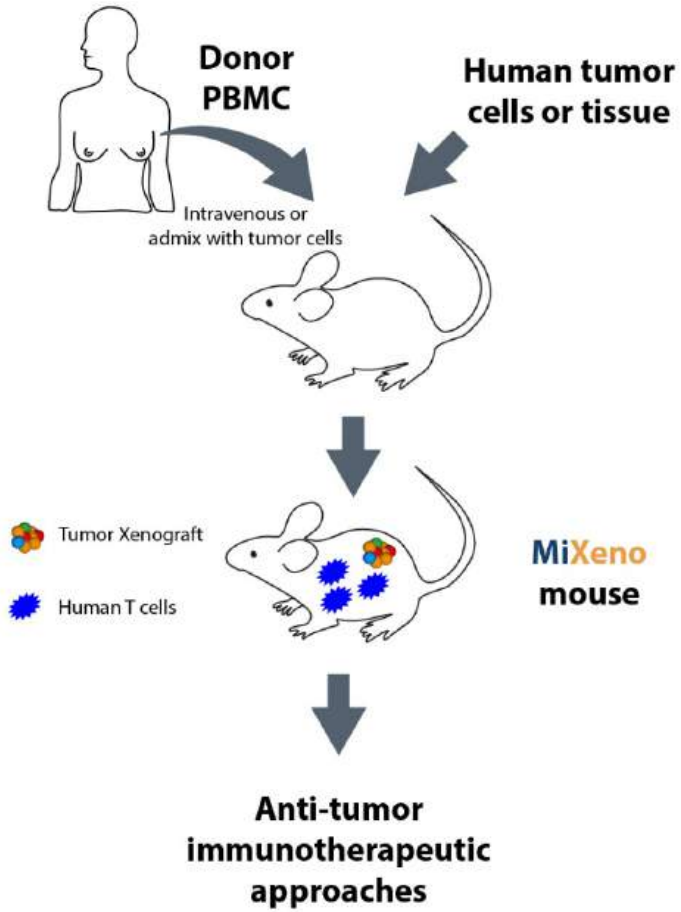


XenoBase

OncoExpress

- **Free online database for curated CDX models**
- **Over 2,000 cell lines** available
- Combining public **profiling data** (gene expression, copy number and mutation) and proprietary ***in vivo* pharmacology data**
- Make an informed decision by searching based on gene mutation, amplification and expression, tumor growth *in vivo* and response to SoC treatments

MiXeno Concept



Applications of MiXeno

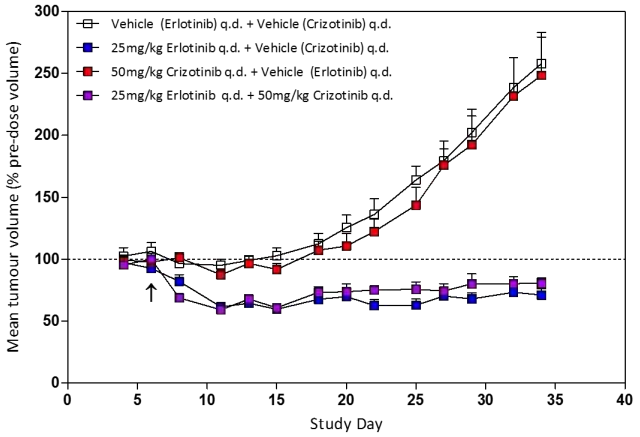
Immune Function Involved	Test Substance	CrownBio Experience
T cell function	BiTE	CD-19, HER2, EGFR BiTEs
	Immune checkpoint inhibitors/agonists	PD-1, PD-L1 inhibitors
	CAR-T	On-going
NK cell function	ADCC	Cetuximab
	NK modulating agents	N/A

A fast and economic way to evaluate I/O therapies alone or in combination

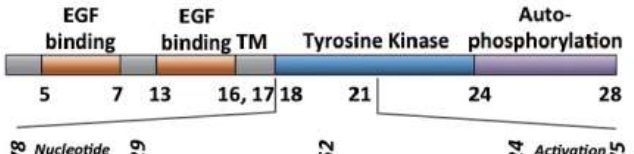
HCC-827 NSCLC model

- Somatic mutations in EGFR gene in NSCLC
- HCC-827 NSCLC adenocarcinoma cell line
- Harbours an activating EGFR mutation (del E746-A750)
- HCC-827 erlotinib resistant models generated with c-met amplification
- High PD-L1 expression (Xenobase & FACS)

HCC-827 WT model shows exquisite sensitivity to Erlotinib whereas as the resistant model shows c-met amplification and sensitivity to Crizotinib



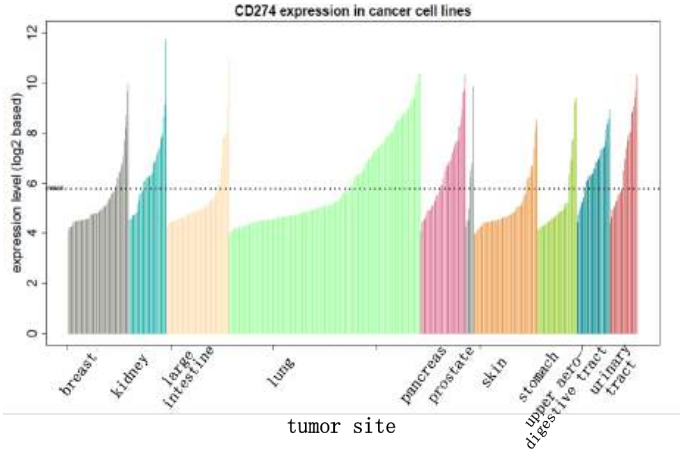
Somatic Mutations in the *EGFR* Gene

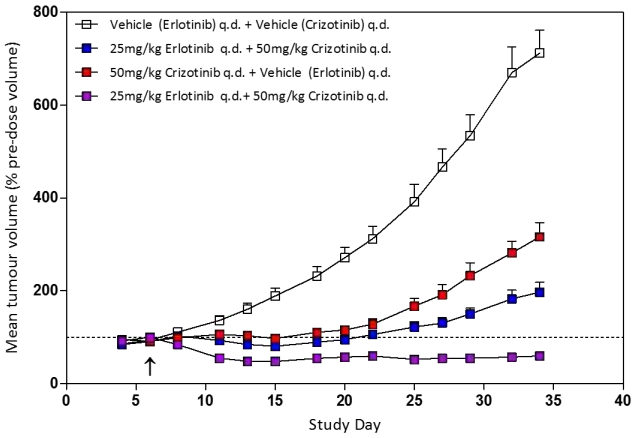


EXON 18	EXON 19	EXON 20	EXON 21
G719X (3%)	LREA deletion (45%)	V765A (<1%)	L858R (40%)
	VAIKEL insertion (1%)	T783A (<1%)	L861X (2%)
	L747S (<1%)	V774A (<1%)	T854A (<1%)
	D761Y (<1%)	S784P (<1%)	A871E (<1%)
		T790M *	
		Exon 20 insertion (4%)	
		V769M (<1%)	
		V769M (<1%)	

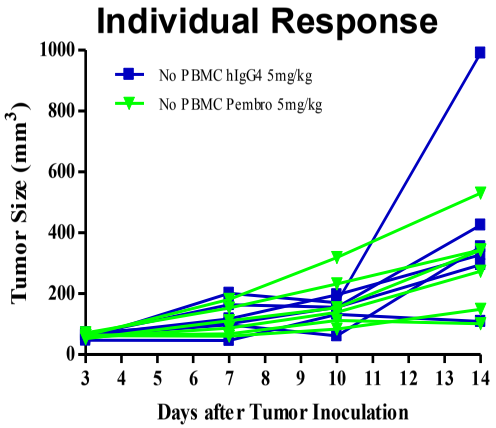
Transl Lung Cancer Res. 2015 Feb;4(1):67-81.

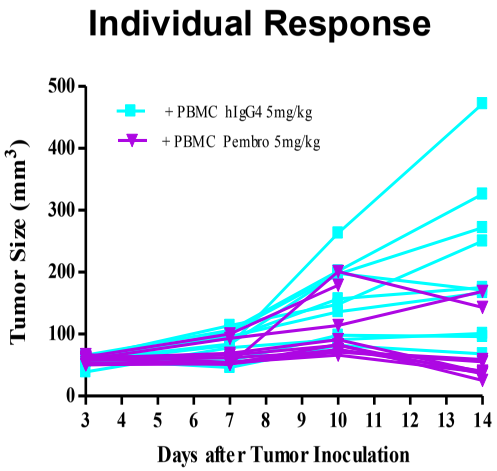
PD-L1 expression



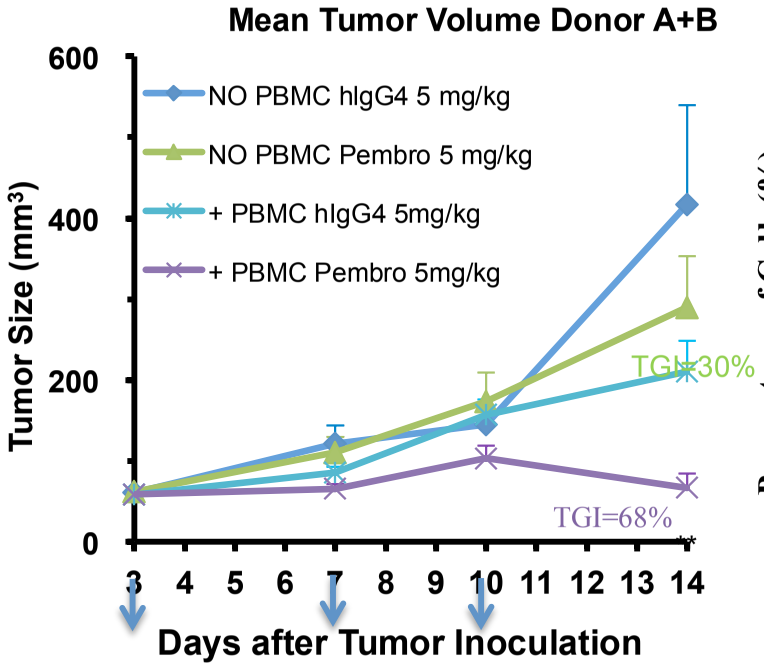


HCC827 – MiXeno Model Anti-PD-1 Ab Antitumor Activity

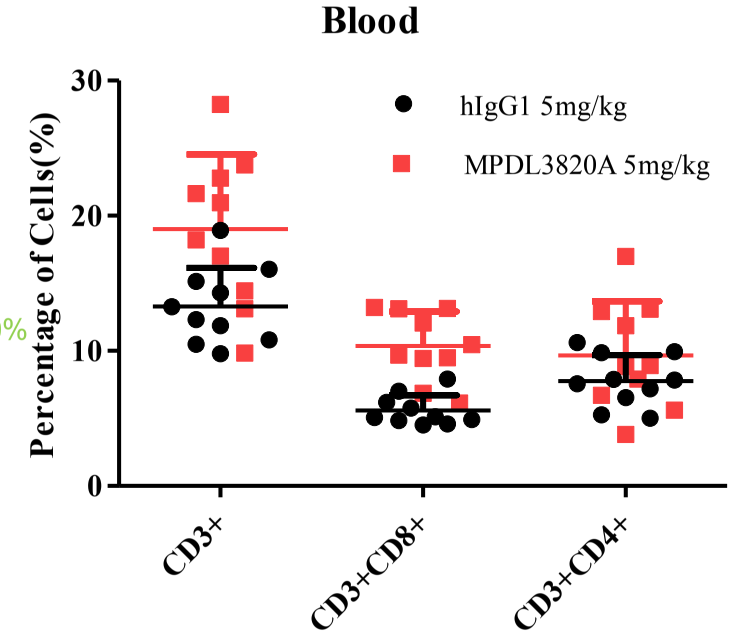




Pembrolizumab demonstrated significant antitumor activity in HCC827 MiXeno model



Increased lymphocyte presence after treatment with anti-PD-L1 antibody

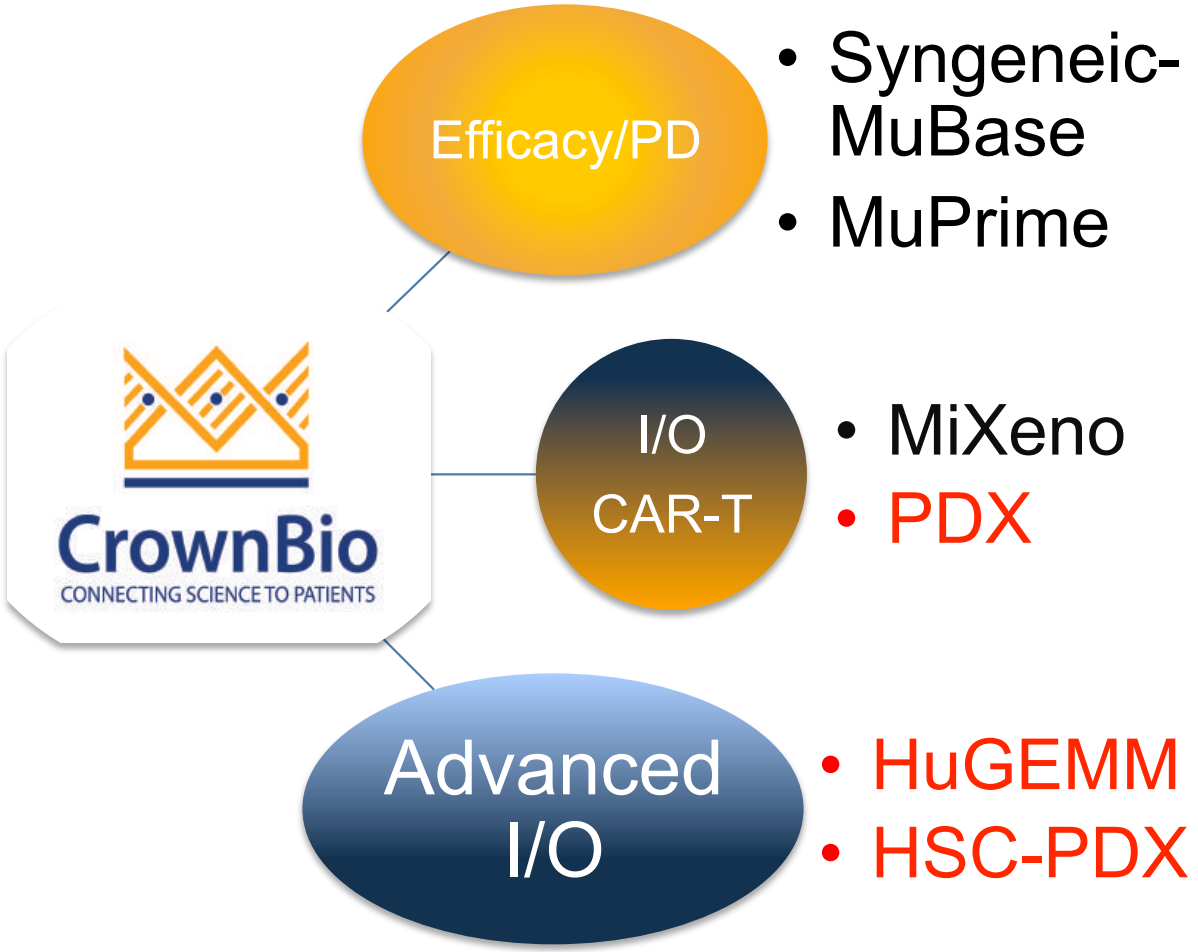


- Evaluation of combination & response in resistant model underway
- PDX models with ERGFR mutations

Zhang et al 2016 AACR 2016 New Orleans

Additional models/developments

Global I/O Platform Technologies



PDX Oncology Knowledge Databases

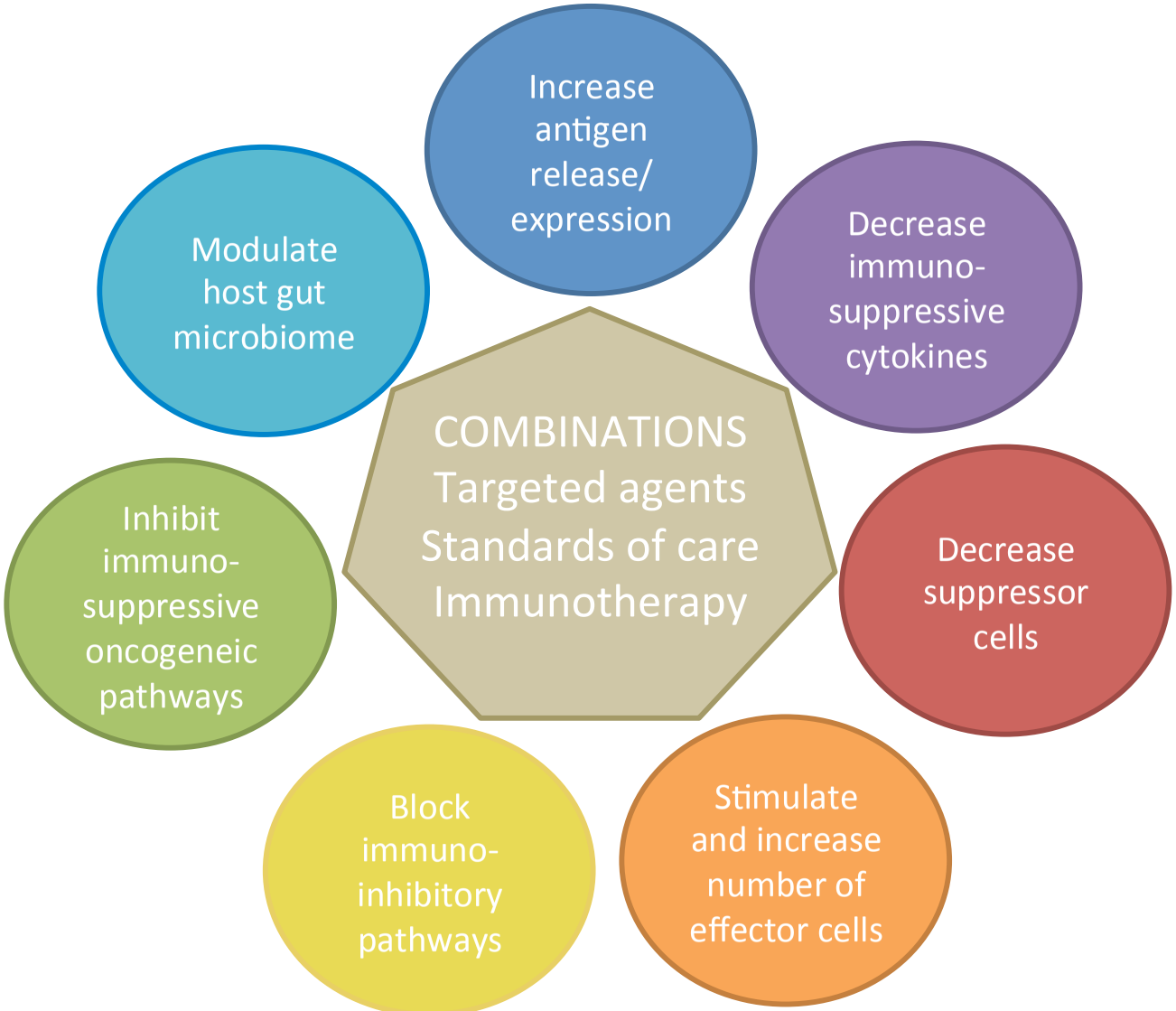
OncoExpress

HuBase2®

HuBase

- **Free online database to access our curated >2500 PDX models**
- **Complete genomic annotation** (gene expression, copy number and mutation), **clinical and pharmacology data**
- Select models that match your criteria using: clinical information, diagnosis and pathology, whole genome gene expression profiling and copy number variation, Hotspot sequence mutation, key biomarker IHC information, tumor growth and SoC response curve

Summary: Strategies to induce anti-tumour immune response



CrownBio
CONNECTING SCIENCE TO PATIENTS

Thank you & acknowledgments

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Tommy Broudy
Jayant Thatte

CBNC
Leo Liu

CBUK
Rajendra Kumari*
Andrew McKenzie
Nektaria Papadopoulou

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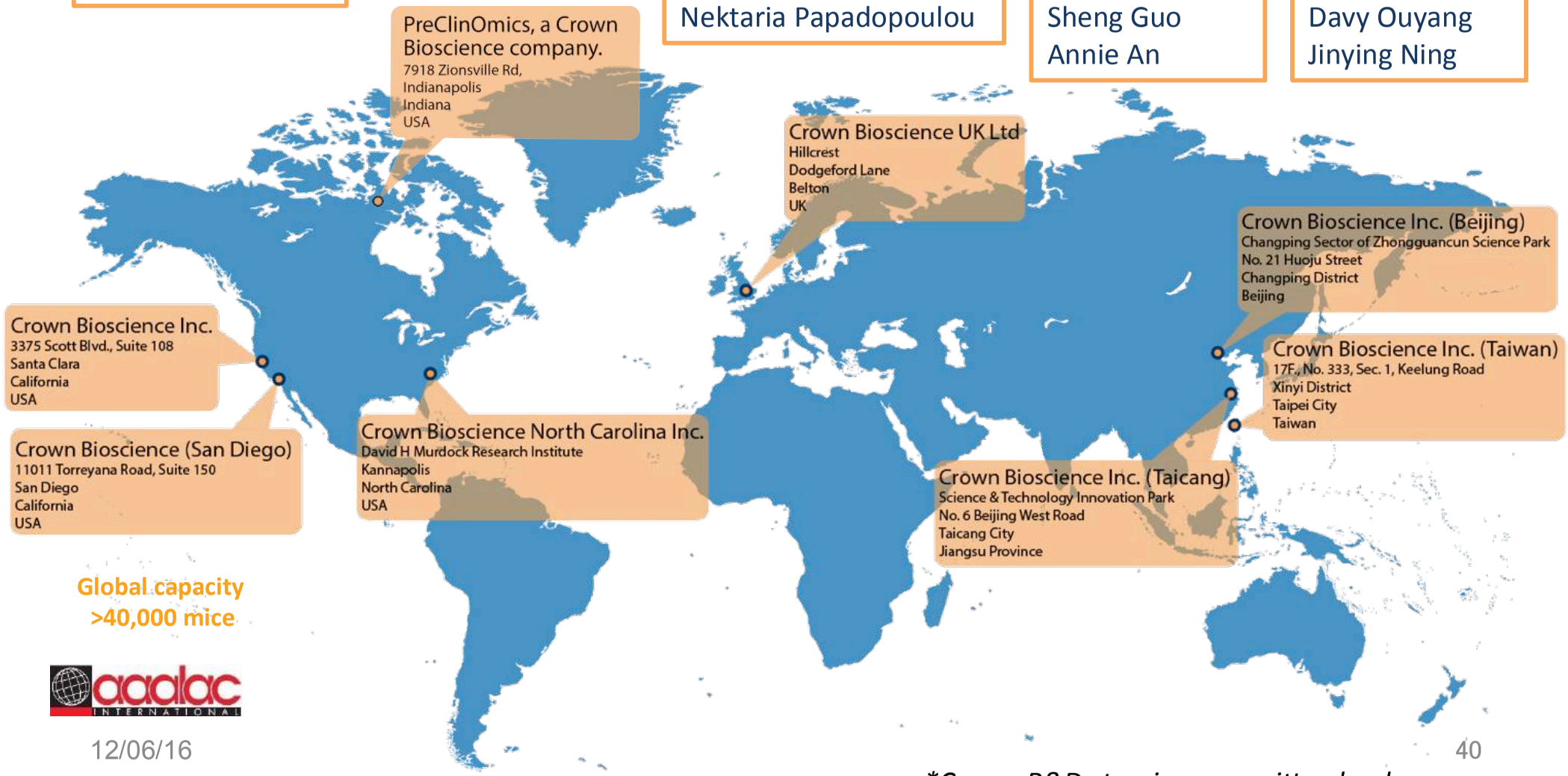

Global capacity >40,000 mice

aalac INTERNATIONAL

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