VALUE OF NEW SPONTANEOUS AND CARCINOGEN-INDUCED MOUSE-DERIVED ISOGRAFT (MDI) TUMOR MODELS FOR DRUG DEVELOPMENT OF NOVEL IMMUNE THERAPEUTIC APPROACHES

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Introduction

Checkpoint inhibitor treatment has already become a common therapy of various cancer types. However, there is still a growing need for well-characterized preclinical mouse models, as clinical data indicate that patients only partially respond to this regiment. Currently, cell lines cultured from the 1970s are used frequently to evaluate novel therapies.

To this end, new mouse-derived isografts (MDI) were established from spontaneously occurring (JA-0009) or carcinogen-induced tumors (JA-2011 and JA-2042). These MDI tumors are transplanted as tissue pieces in a PDX-like manner from animal to animal and are tested for their solid growth. Furthermore, the efficacy of immune checkpoint inhibitor treatment is evaluated and the presence of different immune cell populations in the tumor is characterized by flow cytometry analysis. In addition, RNA-seq data complete the first characterization of these models and will give insights into the expression level and genetic modification of genes in these models.

Results

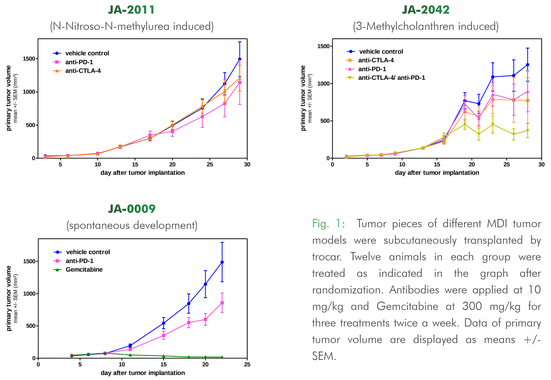

Fig. 1: Tumor pieces of different MDI tumor models were subcutaneously transplanted by trocar. Twelve animals in each group were treated as indicated in the graph after randomization. Antibodies were applied at 10 mg/kg and Gemcitabine at 300 mg/kg for three treatments twice a week. Data of primary tumor volume are displayed as means +/- SEM.

Results

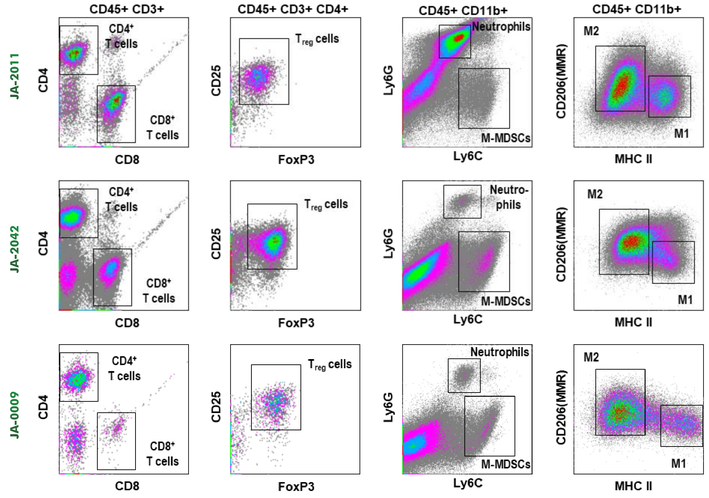

Fig. 2: Cells isolated from solid tumors of the vehicle group were stained for MDSC, T cell and Macrophage markers and analyzed by flow cytometry. Example flow images are shown for each indicated MDI tumor with additional markers given above each graph.

Results

Table 1: Expression of tyrosine-kinase receptors in JA-0009, JA-2011 and JA-2042 tumors.

Gene	Gene description	Gene expression (FPKM values)					
		JA-0009		JA-2011		JA-2042	
		sample 1	sample 2	sample 1	sample 2	sample 1	sample 2
Kdr	kinase insert domain protein receptor, VEGF-R2	8.579	10.474	7.432	13.084	6.298	8.009
Fgfr1	fibroblast growth factor receptor 1	122.697	138.217	166.383	137.789	112.092	118.213
Fgfr2	fibroblast growth factor receptor 2	1.146	0.031	4.227	2.491	0.937	1.072
Pdgfra	platelet derived growth factor receptor, beta polypeptide	40.534	34.803	60.614	51.228	62.505	72.651
Met	met proto-oncogene	120.096	83.597	24.139	30.064	33.087	36.542
Ret	ret proto-oncogene	0.225	0.179	1.058	0.653	0.076	0.084
Flt3	FMS-like tyrosine kinase 3	0.378	0.276	0.240	0.292	1.259	1.437
Axl	AXL receptor tyrosine kinase	172.031	163.433	203.023	210.440	261.356	242.636
Kit	KIT proto-oncogene receptor tyrosine kinase	2.162	1.856	4.323	5.736	5.162	6.012
Egfr	epidermal growth factor receptor	7.592	7.015	9.604	11.132	13.700	15.918
Alk	anaplastic lymphoma kinase	0.087	0.166	0.010	0.021	0.073	0.112
Ephb4	Eph receptor B4	28.023	25.817	13.665	10.399	22.000	24.307
Erbb2	erb-b2 receptor tyrosine kinase 2	7.034	6.063	9.046	7.539	7.018	6.076
Erbb4	erb-b2 receptor tyrosine kinase 4	0.000	0.000	0.000	0.000	0.000	0.000
Igf1r	insulin-like growth factor I receptor	9.052	9.236	8.474	7.896	8.069	8.482
Mst1r	macrophage stimulating 1 receptor (c-met-related tyrosine kinase), RON	1.309	0.488	4.416	3.608	1.397	0.656
Tek	TEK receptor tyrosine kinase, TIE2	3.116	4.038	12.923	14.449	1.985	2.598
Flt4	FMS-like tyrosine kinase 4, VEGF-R3	2.852	3.971	3.777	5.235	2.801	4.222

Table 2: Expression of immune population markers (T, B, NK cells, macrophages, myeloid cells) in JA-0009, JA-2011 and JA-2042 tumors.

Gene	Gene description	Gene expression (FPKM values)					
		JA-0009		JA-2011		JA-2042	
		sample 1	sample 2	sample 1	sample 2	sample 1	sample 2
Ptprc	protein tyrosine phosphatase, receptor type, C, CD45	37.323	30.111	16.357	15.704	30.151	28.046
Cd19	CD19 antigen	0.017	0.086	0.025	0.000	0.245	0.111
Cd3d	CD3 antigen, delta polypeptide	0.793	0.873	0.447	0.267	3.658	3.015
Cd4	CD4 antigen	1.952	0.759	1.971	0.965	33.392	30.082
Cd8b1	CD8 antigen, beta chain 1	0.608	0.404	0.875	0.346	4.468	4.068
Foxp3	forkhead box P3	0.124	0.117	0.309	0.123	0.685	0.587
Cd44	CD44 antigen	217.964	154.191	80.282	88.770	113.863	115.516
Il2ra	interleukin 2 receptor, alpha chain, CD25	0.708	0.449	0.248	0.112	0.734	0.689
Cd51b	integrin alpha M	59.742	47.742	33.733	44.577	22.264	21.607
Adgre1	adhesion G protein-coupled receptor E1, F4/80	35.325	30.841	25.396	23.390	75.824	67.878
Mrc1	mannose receptor, C type 1, CD206	40.398	32.636	70.140	91.794	95.140	82.647
Itgax	integrin alpha X, CD11c	7.283	3.457	3.435	3.349	7.650	7.708
Ncr1	natural cytotoxicity triggering receptor 1, NKp46	4.617	3.767	0.354	0.286	2.626	1.900
Itgb2	integrin alpha 2, CD18b	5.220	3.428	1.137	1.731	1.189	1.887
Lyl1	lymphocyte antigen 6 complex, locus C1	303.057	337.000	41.151	51.858	41.983	36.347

Table 3 Expression of IFN-γ signature in JA-0009, JA-2011 and JA-2042 tumors.

Gene	Gene description	Gene expression (FPKM values)					
		JA-0009		JA-2011		JA-2042	
		sample 1	sample 2	sample 1	sample 2	sample 1	sample 2
Il2rg	interleukin 2 receptor subunit gamma	42.246	39.482	19.466	20.503	62.764	49.006
Cxcr6	chemokine (C-X-C motif) receptor 6	1.257	0.773	1.433	0.318	9.097	6.613
Cd3d	CD3 antigen, delta polypeptide	0.793	0.873	0.447	0.267	3.658	3.015
Cd2	CD2 antigen	1.575	0.898	0.597	0.706	4.033	2.505
Itgal	integrin alpha L	6.752	4.789	3.783	3.901	12.127	11.312
Tagap	T cell activation Rho GTPase activating protein	4.636	5.536	4.018	3.987	7.021	7.502
Ciita	class II transactivator	0.161	0.136	0.010	0.513	14.236	12.366
Ptprc	protein tyrosine phosphatase, receptor type, C	37.323	30.111	16.357	15.704	30.151	28.046
Cxcl9	chemokine (C-X-C motif) ligand 9	4.238	2.831	1.393	0.923	45.016	24.324
Ccl5	C-C motif chemokine ligand 5	41.223	36.996	7.127	7.141	79.711	65.278
Nkg7	natural killer cell group 7	7.640	7.409	2.421	1.104	21.513	20.296
Gzma	granzyme A	24.492	15.757	1.515	1.445	2.996	3.072
Prf1	perforin 1 (pore forming protein)	10.770	6.110	0.600	0.709	2.331	2.125
Ccr5	chemokine (C-C motif) receptor 5	63.335	62.056	15.800	17.280	30.596	28.809
Cd3e	CD3 antigen, epsilon polypeptide	0.818	0.675	1.223	0.438	7.494	6.838
Gzmk	granzyme K	0.940	0.437	0.290	0.000	0.587	1.035
Ifng	interferon gamma	0.039	0.099	0.112	0.000	1.273	0.848
Gzmb	granzyme B	26.809	12.568	0.783	0.792	1.399	0.905
Pdcd1	programmed cell death 1	1.520	1.512	0.676	0.380	6.870	5.895
Slamf6	SLAM family member 6	2.433	2.073	1.518	0.929	3.694	3.940
Cxcl13	chemokine (C-X-C motif) ligand 13	26.792	6.980	0.176	0.566	2.667	0.784
Cxcl10	chemokine (C-X-C motif) ligand 10	78.838	79.382	8.332	6.334	19.421	10.466
Ido1	indoleamine 2,3-dioxygenase 1	0.771	1.136	0.128	0.000	0.033	0.000
Lag3	lymphocyte-activation gene 3	3.108	2.039	2.392	1.374	8.097	6.592
Stat1	signal transducer and activator of transcription 1	44.665	38.086	11.567	10.864	31.248	24.513
Cxcl11	chemokine (C-X-C motif) ligand 11	14.251	11.497	0.686	0.563	2.396	0.793
Il12b	interleukin 12b	0.067	0.042	0.072	0.026	1.628	1.269
Il12a	interleukin 12a	0.218	0.092	0.052	0.056	0.119	0.000
Irf1	interferon regulatory factor 1	53.329	49.470	66.608	44.893	108.433	98.369
Tbx21	T-box 21	0.344	0.327	0.118	0.177	1.057	0.906
Ctla4	cytotoxic T-lymphocyte-associated protein 4	0.491	0.209	0.161	0.103	0.879	0.632
Cd274	CD274 antigen, PD-L1	6.746	4.850	3.991	4.090	4.693	2.803

Description: Tumor pieces of JA-0009, JA-2011 and JA-2042 were used for total RNA preparation of two samples for each tumor. Sequencing of 150 nt fragments with 25 million reads was performed with Illumina Next Seq 500. FPKM (fragments per kilobase million) were calculated and are depicted for a number of genes.

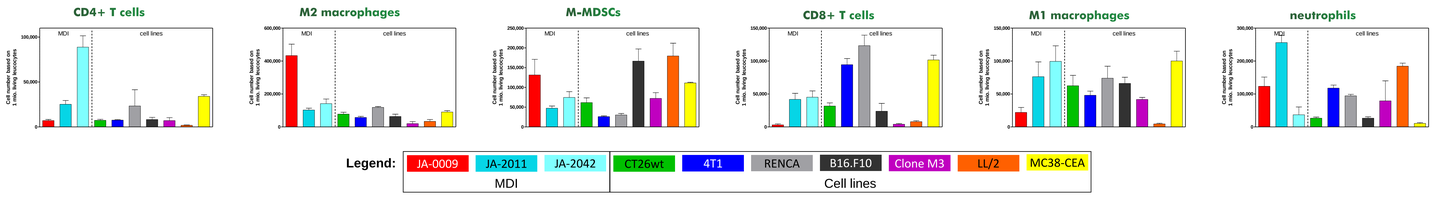

Fig. 3: Cells isolated from solid tumors of the vehicle group were stained for MDSC, T cell and macrophage markers and analyzed by flow cytometry. The graphs depict the number of cells per 1 million leukocytes in the indicated tumors (see legend). For the comparison of the results, the data for the cell line-derived tumors are shown.

Summary

- Summary
- Development of new syngeneic models with robust growth
- Syngeneic models respond different to immune checkpoint inhibitors
- Preservation of tumor phenotype via propagation from animal to animal
- Correlation of RNAseq with flow cytometry data (e.g. high T cell proportion in JA-2042 tumor)
- Higher expression of genes of the IFN-γ signature correlates with checkpoint inhibitor efficacy

Outlook

- Further carcinogen-induced models are offered: JA-2019 and JA-2041
- More spontaneous arosen tumors are under development: JA-0017, JA-0023 and JA-0032

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