SUBQPERIOR: INTRA-MAMMARY FAT PAD IMPLANTATION AS AN ALTERNATIVE IMPLANTATION METHOD FOR SYNGENEIC TUMOR MODELS

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Introduction

Syngeneic tumor models used for discovery of immune therapeutics should have several features such as a long study duration, equal responsiveness to checkpoint inhibitors from study to study and a high homogeneity in tumor growth. Moreover, models should consider the ethical rules (3R reduce, refine, replace). At present, the standard implantation method for syngeneic tumor models is the subcutaneous tumor cell inoculation (s.c.).

We have developed an alternative implantation method for syngeneic tumor models: the inoculation into the mammary fat pad (intramammary, i.ma.). Both implantation sides are heterotopic related to the original tumor entity except for syngeneic breast tumor cells. In addition, both tumor inoculation methods can easily be applied and monitored by calipering reducing the costs.

Summary of tumor parameters from establishment studies

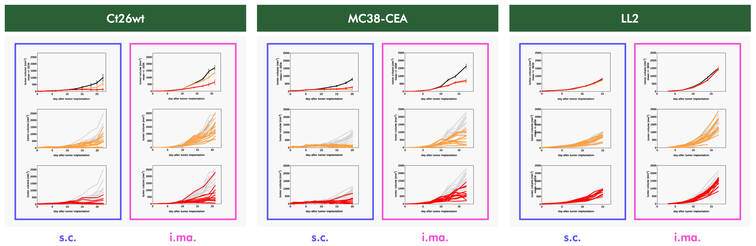
Tumor entity	Cell line	Study duration (d)		final tumor volume (mean)		tumor ulcerations (n)	
		s.c.	i.ma.	s.c.	i.ma.	s.c.	i.ma.
BREAST	4T1	14	30	240 mm³	1410 mm³	10	3
	EMT-6	11	18	290 mm³	1360 mm³	8	1
COLON	Ct26wt	18	18	670 mm³	2080 mm³	4	0
	MC38-CEA	21	23	300 mm³	2370 mm³	4	0
KIDNEY	RENCA	18	23	240 mm³	1230 mm³	3	0
LIVER	Hepa1-6(Z1)	n.a.	40	n.a.	1160 mm³	n.a.	0
LUNG	AB12	42	65	360 mm³	1130 mm³	4	0
	LL2	16	18	780 mm³	2210 mm³	2	0
MELANOMA	B16F10	14	16	680 mm³	2510 mm³	1	0
	CloneM3(Z1)	16	21	470 mm³	1620 mm³	3	0

Summary of efficacy study parameters calculated from the establishment study results. Efficacy study end was assumed when >33% of animals (5th mouse of 12) were euthanized due to ethical abortion criteria, e.g. tumor ulceration or size. Efficacy study parameters include study duration, final tumor volume and number of animals, which were euthanized because of tumor ulceration until assumed efficacy study termination.

Method

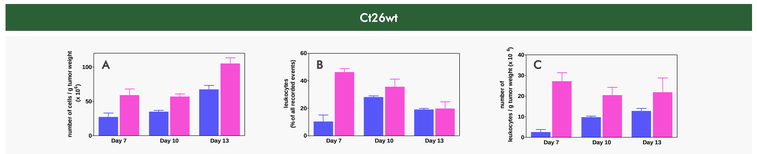
Method Ten murine tumor cell lines from various entities were inoculated either into the mammary fat pad or the subcutaneous space in their syngeneic mouse background in 12 mice each and tumor parameters compared. Tumor development was monitored by calipering. Mice were euthanized in accordance with the GV SOLAS (Germany) guidelines in case of occurrence of tumor ulceration, tumor diameter or other ethical abortion criteria.

Efficacy studies using anti-mPD-1 and anti-mCTLA-4



Efficacy studies using ICIs. Evaluation of the antitumoral efficacy of anti-mPD-1 and anti-mCTLA-4 treatment in CT26wt, MC38-CEA and LL2 tumor models. The tumor cells were implanted subcutaneously (s.c.) or into the mammary fat pad (i.ma.). Mice were treated 3 times with immune-checkpoint inhibitors at 10 mg/kg intraperitoneally. Mean tumor volume and single growth curves are depicted.

Time-dependent leukocyte content in CT26wt tumors

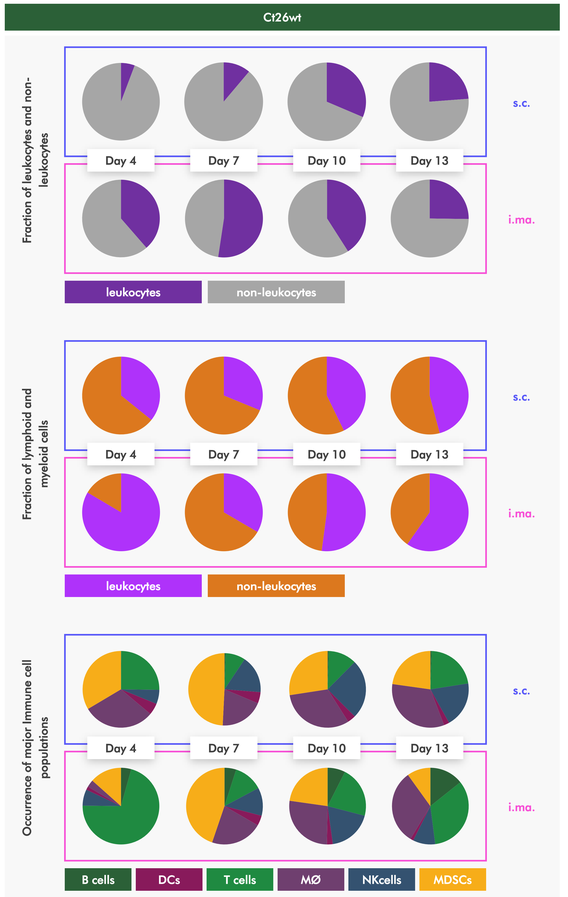


Leukocyte content. On Day 0, syngeneic CT26wt colon tumor cells were implanted subcutaneously (s.c.) or into the mammary fat pad (i.ma.). On Days 7, 10 and 13 after implantation five animals were euthanized and the tumor harvested for flow cytometry analysis: **the number of cells per g tumor mass was calculated (A). The percentage of living leukocytes (CD45+ cells) of all recorded events was determined (B) and the number of leukocytes per g tumor mass was calculated (C).**

Method

Method For flow cytometry analysis tumor material was disrupted, erythrocytes removed, and the isolated single cell suspensions counted. Thereafter, the number of cells per g tumor mass was calculated (A). The single cells were stained for live/dead and the antigens CD3, CD4, CD8a, CD45, CD25, CD11b, Ly6C, Ly6G, F4/80, CD11c, MHC class II, CD206, CD335, CD49b, B220 and FoxP3. Samples were analyzed by flow cytometry using a LSR Fortessa (Becton Dickinson).

Immune cell distribution in PBS treated tumors



i.ma. tumor models

- Easy to implant
- Can be calipered
- No tumor ulceration
  - in accordance to 3Rs (refine)
- Higher homogeneity in tumor growth
  - In accordance to 3Rs (reduce)
- Higher final tumor volumes
- Enlarged therapeutic window
- Improved tumor development (e.g. Hepa1-6)
- Similar responsiveness to ICIs
- Higher immune cell infiltration

SubQperior

(intra-mammary tumor application)

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