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<b>Date:</b> 8.11.2022	<b>Scoring and withdrawal criteria of animals bearing i.c. tumors</b>	<b>LTK- RES-5-H-EN</b> <b>Version: H</b>
<b>This SOP replaces:</b> Version: G		
<b>Reason for Change:</b> Response to veterinary office		
<b>Related SOPs:</b>	SOP-LTK-TRT-13 Isoflurane anesthesia SOP-LTK-TRT-18 Injection anesthesia SOP-LTK-RES-6 Bioluminescent in vivo imaging SOP-LTK-TRT-19 Tail bleeding SOP-LTK-RES-4 Implantation of osmotic minipumps SOP-LTK-TRT-14 CO2 Euthanasia SOP-LTK-RES-43 Buprenorphine analgesia	
<b>Indication of Use:</b>	Animals bearing intracranial tumors	
<b>Aim of SOP:</b>	Assess the wellbeing of animals bearing intracranial tumors after orthotopic injection of syngeneic tumor cells	
<b>Distribution:</b>	<ol style="list-style-type: none"> <li>1. Server</li> <li>2. Animal House</li> <li>3. Group vom Berg</li> </ol>	
<b>Attachments:</b>	<b>Scoring sheet</b>	
Generated at: 08.11.2022	Checked and approved at: 08.11.2022	
by: Sereina Deplazes	by: Michal Beffinger	

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**Responsible Persons:**

- 1) The researcher mentioned on the respective scoring sheet
- 2) Any person with Module 1 and registered on animal permit

**Method:** observation, weighing, handling on cage grid

**Principle of Method:**

**Clinical scoring and observation**

GL-261 and derivatives:

You have to score tumor-bearing mice **weekly until three weeks after tumor-inoculation** (during this time you still have to visually check them 3 times per week), **then daily** when you inject **up to 50'000 cells**.

**Attached scoring sheet is laid out for GL-261-bearing animals. If not GL-261, adjust days and select which non-GL-261 tumors have been inoculated (see below)**

SMA560 and derivatives

You have to score tumor-bearing mice **once a week** until **one week after tumor inoculation** (during this time you still have to visually check them 3 times per week), **then daily** when you inject **up to 50'000 cells**.


SB28 (PDGF+ p53<sup>-/-</sup> neural progenitor derived tumor cells) and derivatives

You have to score tumor-bearing mice **on day 1 and day 4** (during this time you still have to visually check them 3 times per week), **then daily from day 5 on** when you inject **up to 50'000 cells**.

**This INCLUDES checking on both days of the weekend.**

For scoring you have to

- 1) **weigh** the animals. The mouse continues to gain weight over the time of the experiment. Weigh the animal and calculate the weight loss compared to the peak weight of the experiment (you can define the peak weight as soon as you see a stop in weight gain).
- 2) **observe** their behavior in a familiar environment in the cage without manipulation: gait, grooming, social behavior, performance, slight paralysis, etc.
- 3) **observe** their behavior in a non-familiar environment on the grid of a cage and on the balance during weighing: gait, missed steps, performance, balance, explorative behavior, slight paralysis, etc. A non-impaired healthy mouse does immediately start to explore its new surroundings (cage grid, balance). An impaired mouse will stay where placed on the balance or the grid and will not start to move spontaneously.

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**Units and Formulas:** weight in grams, Severity grades

**Material to be used:** home cage, balance, cage grid

**Min/Max amount:** Severity grades ranging from 0-3 are being used in this protocol, where 0 is no impact on the animals' well-being, and 3 is strong impact leading to immediate withdrawal and euthanasia.

**Safety:**

1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Only in the case of chemotherapeutic agents and human cytokines additional biosafety rules have to be obeyed
3. Follow the rules of the animal house

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**Method Description:**


Score	One of the following clinical signs
0	No visible impairment, normal gait and behavior including explorative behavior in new environment (grid, weighing scale), no steps missed on the grid
1	Mild impairment, normal gait and behavior including spontaneous explorative behavior in new environment (grid, weighing scale), occasional step missed on the grid (less than one third of steps missed), slight balance and coordination impairments, slightly reduced activity, age characteristic weight gain absent / delayed
2	Reduced activity, less motile than healthy animals, reduced explorative behavior (when placed on balance or grid, no immediate exploring), signs of discomfort (transient hunched back position, lack of grooming), reduced performance on grid / over one third of steps are missed, 15 - <20% weight loss compared to peak weight
3	Severe ataxia/apathy, no more activity, signs of stress and discomfort, weight loss of $\geq 20\%$ of peak weight (withdrawal criterium) OR ANY OF THE IMMEDIATE WITHDRAWAL CRITERIA below

If **any** of the symptoms for clinical **score 1** or higher are present, immediately switch to daily scoring. Add 50% breeding chow to the normal chow, offer small pieces of wet gel food.

**Score 2:** Add 50% breeding chow to the normal chow and add wet gel food. Apply analgesia for up to three days (SOP-LTK-RES-43 Buprenorphine analgesia), **Score 3:** Euthanize animals immediately (SOP-LTK-TRT-14 CO2 Euthanasia).

**Immediate Withdrawal criteria:**

- Sacrifice mice immediately when any of the following signs are visible:  $\geq 20\%$  weight loss / score 3
- Seizures or generalized spasms (visual examination)
- nasal or eye discharge (visual examination)
- Vocalization (auditory examination)
- Respiratory difficulty (visual examination)
- Cranial deformity/neurological signs (visual examination)
- Perianal soiling (visual examination)
- Rough/unkept haircoat (visual examination)
- Hunched posture/Kyphosis in combination with piloerection AND social isolation AND lack of activity (visual examination)
- Skin pathology (visual examination)
- Restricted mobility (visual examination)
- Jaw deformity/malocclusion (visual examination)
- Hypothermia (thermo-sensory examination: touch)

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**Factors influencing outcome:**  
Make sure that animals are weighed at the same time of the day as mice show considerable weight fluctuations depending on resting and feeding periods over 24h

**Analysis:**  
Weight is recorded for every individual day, as soon as weight starts to drop, the maximal (peak) weight is noted in the respective column in the scoring sheet and the corresponding weight after weight loss -15% and -20% is calculated and noted down.

**Documentation:**  
Scoresheets of ongoing experiments in the animal house,  
Finished experiments: Server and folder with respective license nr (to be announced)

**Literature:**  
Gall GA, Kyle WH. Growth of the laboratory mouse. Theor Appl Genet. 1968 Jul;38(7):304-8. doi: 10.1007/BF01297571. PubMed PMID: 24442355.

**Attachment: Scoring sheet (next pages)**

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**Scoring sheet for monitoring intracranial tumors (GL-261)**

- Adapted for PDGF<sup>+</sup>p53<sup>-/-</sup> (Daily scoring 2 weeks after inoculation)
- Adapted for SMA560 (Daily scoring 1 week after inoculation)
- Adapted for SB28 (Score day 1 and 4, daily scoring day 5 after inoculation)

Animal permit #:

Stereotactic inoculation:


Researcher:

Experiment ID:

Cage	Animal ID	Weight [g]			-1 before surgery		1		3		5		7	
		Peak	Peak -20%	Peak -15%	S	T	S	A	T	S	T	S	A	T
		[g]	[g]	[g]	[g]	[g]	[g]	Y/N	[g]	[g]	[g]	[g]	Y/N	[g]
1	1													
	2													
	3													
	4													
	5													
	6													
	...													
	...													
Signature														
Comments														

Continue the scoring until end of experiment (3-5 weeks) or in the case of survival studies until 4 months, add additional sheets if necessary! **This score sheet is used for all animals bearing intracranial tumors!**

Weight [g] and clinical score on **days in bold letters**. For days in *italics* (non bold) visual observation in the homecage is sufficient if no visible impairment can be detected (S – score, [g] – weight in grams, A - analgesia, T – time of day)

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Animal permit #:


Stereotactic inoculation:

Researcher:

Experiment ID:

Cage	Animal ID	Weight [g]			10		12		14		17		19		21		Weight [g] and clinical score on <b>days in bold letters</b> . For days in italics (non bold) visual observation in the homecage is sufficient if no visible impairment can be detected (S – score, [g] – weight in grams, A - analgesia, T – time of day)	
		Peak – 20%	Peak –15%		S	T	S	T	S	T	S	T	S	T	S	T		
1	1																	
2	2																	
3	3																	
4	4																	
5	5																	
6	6																	
...	...																	

Continue the scoring until end of experiment (3-5 weeks) or in the case of survival studies until 4 months, add additional sheets if necessary! **This score sheet is used for all animals bearing intracranial tumors!**

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Animal permit #:

Stereotactic inoculation:


Researcher:

Experiment ID:

Cage	Animal ID		Weight [g]			<i>Weight [g] and clinical score on days in bold letters. For days in italics (non bold) visual observation in the homecage is sufficient if no visible impairment can be detected (S – score, [g] – weight in grams, A - analgesia, T – time of day)</i>														
			Peak	Peak	Peak															
				– 20%	–15%															
1	1				<b>22</b>	S	[g]	A	Y/ N	T										
	2					S	[g]	A	Y/N	T										
		3																		
		4																		
		5																		
		6																		
	...	...																		

Continue the scoring until end of experiment (3-5 weeks) or in the case of survival studies until 4 months, add additional sheets if necessary! **This score sheet is used for all animals bearing intracranial tumors!**



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