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Measures to increase the repeatability and translatability of (early stage) preclinical cancer research

Background:

Alarming recent reports on frequent heterogeneity of results in confirmatory biomedical studies, especially cancer research, oppose an economic and ethical dilemma on society. This obstacle demands for improvements in data management and data reporting. Here we present two measures to do so suitable for an academic lab with limited available resources and frequently changing personnel: Firstly, the introduction an quality management system (QMS) featuring an Open Source electronic lab notebook (ELN) eLabFTW not only improves Open Science character of the working group but- to our experience- also enhances stakeholder engagements particularly for non-full time lab staff and for the management team of a biobank. Secondly, by systematic assessing and meta analyzing the published literature on (a selected theme) of *in vitro* cancer research, we reveal severe limitations in current reporting practices in early stage cancer research and identify the insufficient reporting on nutrient composition of the used cell culture media to present a significant source of heterogeneity of results from replication experiments.

Results:

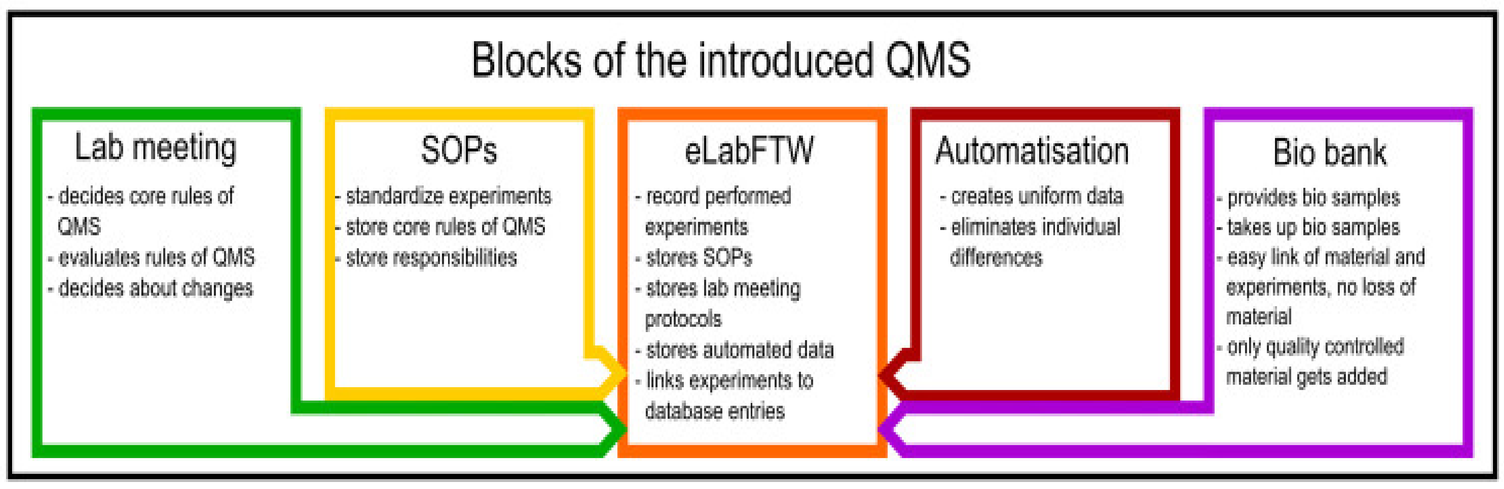

Fig1: Proposal of a QMS suitable for academic lab environment incl. ELN eLabFTW

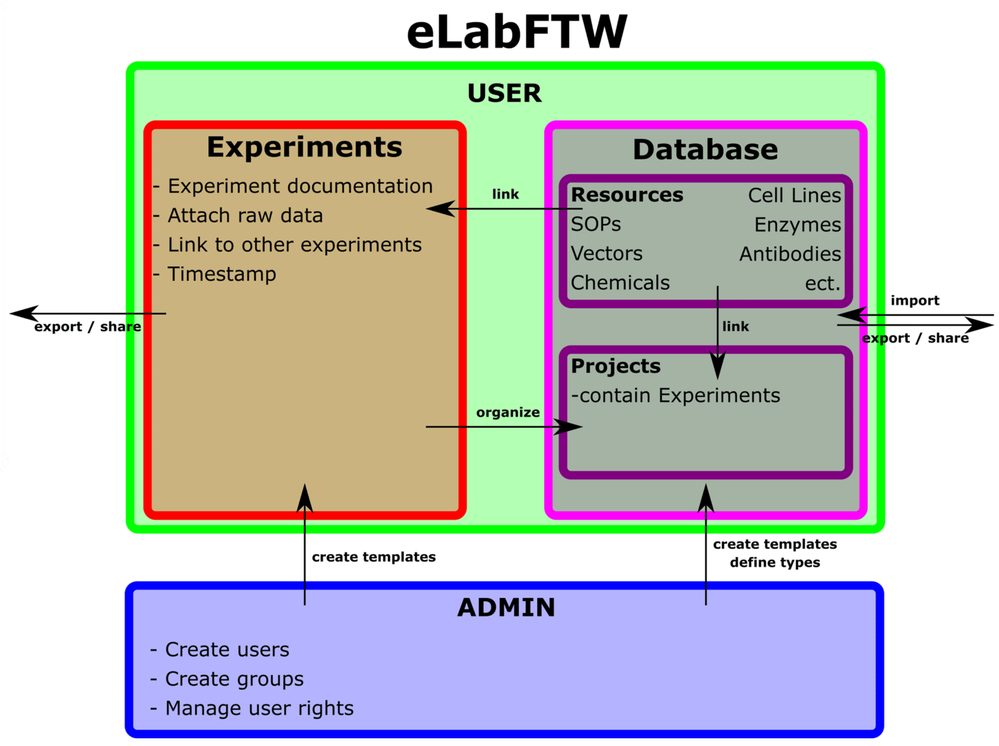

Table 1: Literature screening criteria

Inclusion criteria	Exclusion criteria
U-87 MG cell line as glioblastoma in vitro model	Other models than U-87 MG cell line In vivo models, Xenotransplantation models
TMZ single treatment	TMZ as a part of a combined treatment with other drugs or genetic interventions
Comparison of the effect of TMZ to an untreated control	No comparison to an untreated control
Cell viability assessment (MTT and similar colorimetric assays, cell counting) to quantify the effect of TMZ	Effect of TMZ measured with none of these cell viability assessment methods
DMEM as the cell culture medium	Other cell culture media than DMEM
Original peer-reviewed research articles	Other publication types (e.g., conference abstracts, poster presentations)
English language	Other languages than English

Articles were included if they met all inclusion criteria and no exclusion criteria. If an article included multiple experiments where one or more experiment did not match the criteria but at least one did match, then the article was included. DMEM = Dulbecco's Modified Eagle Medium; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TMZ = temozolomide; U-87 MG = Uppsala-87 Malignant Glioma.

Table 2: Extracted cell concentrations conditions

Cell concentration [cells/µl]	Articles	
5	1	0.7%
12.5 - 62.5	1	0.7%
15	1	0.7%
20	3	2.2%
25	3	2.2%
30	4	2.9%
40	2	1.5%
50	5	3.6%
100	1	0.7%
166.7	1	0.7%
200	1	0.7%
500	1	0.7%
Reporting of only the number of cells per well without the associated volume per well	93	67.9%
No information regarding the cell number, the volume they are plated in or the cell concentration were given	20	14.6%

Table 3: Extracted cell passaging criteria

Criterion	Articles	
Based on cell culture confluence		
50% – 70%	1	0.7%
60% – 80%	1	0.7%
70%	2	1.5%
70% – 90%	3	2.2%
80%	6	4.4%
Based on time intervals		
2 days	1	0.7%
2 – 3 days	1	0.7%
3 – 4 days	1	0.7%
7 days	1	0.7%
No cell passaging criteria were reported	120	87.6%

Table 4: Extracted parameters

Parameter	Phenotype	Articles	
General article information			
Conflict of interests statement	Declaration of no conflict of interests	86	62.8 %
	Declaration of existing conflict of interests	5	3.6%
	No statement about conflict of interests	46	33.6 %
U-87 MG in vitro model			
Source of U-87 MG cells	American Type Culture Collection, Manassas, Virginia	66	48.2 %
	Chinese Academy of Sciences, Beijing, China	27	19.7 %
	Other commercial/institutional sources	24	17.5 %
	Colleagues	11	8.0%
	Not reported	9	6.6%
U-87 MG cell line authentication conducted?	Yes	16	11.7 %
	No/Not reported	121	88.3 %
U-87 MG age (maximum number of cell passage)	3	1	0.7%
	7	1	0.7%
	8	1	0.7%
	10	3	2.2%
	15	4	2.9%
	20	2	1.5%
	35	1	0.7%
	100	1	0.7%
	Not reported	123	89.8 %
U-87 MG culture conditions			
Glucose level of cell culture medium	Low glucose (1000 mg/dl)	3	2.2%
	High glucose (4500 mg/dl)	24	16.8 %
	Low and high glucose (in different experiments)	1	0.7%
	Without glucose	1	0.7%
	Not reported	108	78.8 %
Mycoplasma contamination checked?	Yes	8	5.8%
	Not reported	129	94.2 %
Supplemented antibiotics	Penicillin & Streptomycin	92	67.2 %
	Other antibiotics	5	3.6%
	No antibiotics supplemented	3	2.2%
	Not reported	37	27.0 %
Source of fetal bovine serum (FBS)	Thermo Fisher Scientific, Waltham, Massachusetts (including Gibco, Invitrogen & Life Technologies)	51	37.2 %
	Hyclone Laboratories Inc, Logan, Utah	13	9.5%
	Sigma-Aldrich, St. Louis, Missouri	8	5.8%
	Other sources	22	16.1 %
	FBS was not used	1	0.7%
	Not reported	42	30.7 %
Control group and outcome measurement			
Type of untreated control	Drug vehicle (DMSO)	37	27.0 %
	Cell culture medium only	13	9.5%
	Not reported	87	63.5 %
Cell viability assessment method	MTT assay, colorimetric	67	48.9 %
	Cell Counting Kit-8 (CCK8), colorimetric	20	14.6 %
	Sulforhodamine B (SRB) assay, colorimetric	9	6.6%
	Alamar Blue assay, colorimetric	7	5.1%
	Trypan Blue Exclusion test, cell counting	6	4.4%
	WST-1 assay, colorimetric	6	4.4%
	MTS assay, colorimetric	3	2.2%
	Other assessment methods	11	8.0%
	More than one assay used	8	5.8%

Table 3: Moderators of between-articles-variance of true effects

Moderator	Type	Number of effects	Number of articles	p value	Margi-nal R^2	Between-articles-variance		
						tau^2	I^2	Explained
Without moderators		644	101			2.8%	42.9%	n. a.
U-87 MG source	cat.	644	101	.075	n. s.	2.6%		
U-87 MG authentication	cat.	644	101	.476	n. s.	2.8%		
U87-MG age (Cell passages)	cont.	138	11	.238	n. s.	1.5%		
Cell concentration	cont.	113	20	.323	n. s.	3.8%		
Confluence level at cell passaging	cont.	57	11	.319	n. s.	0.8%		
Glucose level of culture medium	cat.	644	101	.016	7.0%	2.5%[a]	40.1%	10.9%
Mycoplasma exclusion	cat.	644	101	.491	n. s.	2.8%		
Supplemented antibiotics	cat.	644	101	.094	n. s.	2.6%		
FBS source	cat.	644	101	.067	n. s.	2.6%		
Type of untreated control	cat.	644	101	.370	n. s.	2.8%		
Articles reporting quality	int.	644	101	.031	3.3%	2.6%[b]	41.7%	5.0%
TMZ conc.	cont.	644	101	< .001	38.6%	3.4%[c]	64.3%	0.0%
Treatment duration	cont.	644	101	< .001	6.0%	2.9%[d]	45.7%	0.0%

Table 4: Multivariable meta-regressions

			Within-articles-variance		Between-articles-variance	
Moderators	p value	Marg. R^2	tau^2	adjusted I^2	tau^2	adjusted I^2
Without moderators			3.6%	56.6%	2.8%	42.9%
TMZ concentration & treatment duration	< .001	42.1 %	1.7% [1.5%,1.9 %]	30.9%	3.7% [2.7%,5.2%]	68.5%
TMZ concentration & Treatment duration & mediums glucose level	< .001	45.4 %	1.7% [1.5%,1.9 %]	31.9%	3.5% [2.6%,5.0%]	67.4%
TMZ concentration & Treatment duration & articles reporting quality	< .001	44.1 %	1.7% [1.5%,1.9 %]	31.6%	3.6% [2.6%,5.0%]	67.8%
TMZ concentration & Treatment duration & mediums glucose level & articles reporting quality	< .001	45.9 %	1.7% [1.5%,1.9 %]	32.0%	3.5% [2.5%,5.0%]	67.4%

- Systemic review and meta analysis of in vitro research literature is a powerful tool to promote aspects of research integrity and support transformation of wrongful research habits.
- Pre registration also for non-animal research or systematic reviews seems to be a promising option to reduce risk of bias and minimize unintended repetition experiments

SYRF Systematic Review Facility

PROSPERO International prospective register of systematic reviews

References:

Hewera et al., 2020 &2021; Sander et al., 2022