Mutations in the DNA encoding for isocitrate dehydrogenases (IDH) are established molecular markers in 70-80% of stage II or III in glioblastoma. Among IDH1 mutations, R132H is the most common mutation in glioma (90%). IDH1-R132H is expressed in other cancers so the visualization of the dynamics of the cancerous cells within the brain, and their lineage tracking using an animal model could help as an early predictor for grades 2 and 3 glioma. There have been various promising experiments on mice regarding vaccinations, or immunizations targeting IDH1-R132H. Glioblastoma which is the most aggressive form of glioma is a fast-spreading form of glioma, understanding metastasis and migration in these cancer cells is a critical step towards better therapeutics.

Biological question: The aim of the research will consist in 1) develop a cancer brainbow animal model expressing IDH1^{R132H} in the brain subventricular zone (SVS) to visualize expansion and spread of the oncogene IDH1^{R132H} 2) investigate cell migration in term of speed and diffusion across tissues 3) characterize the dynamics of single-cancer cell.

- IDH1 localizes in the cytoplasm and peroxisome, and promotes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG) that protects the cells from reactive oxygen species (ROS) which can cause DNA damage. Mutation in IDH1 forces the cells to convert α-KG into the D isomer of 2-hydroxyglutarate (D2HG). A high concentration of D2HG is toxic for the glioma cells and induces biological alternations like causing oxidative stress, reduction of pro-inflammatory signalling and inhibition of expression of pro-apoptotic proteins.
- We will use the study by Bardella et al. [1] to generate a mouse model of glioma by expressing IDH1-R132H in the subventricular zone (SVZ) in the brains of adult mice. The IDH1$^{fi(R132H)/+}$ knockin mice[1] showed hydrocephalsys and expanded lateral ventricles with an accumulation of D2HG and a reduction of α-KG.
- Once the germline transmission of the mutant allele established, we will engineer and validate a cancer brainbow mouse model. We will follow the methodology detailed in Boone et al. [2]. The cancer rainbow (crainbow) transgene has 4 positions that either express an inert fluorescent protein (position 0), or 3 spectrally resolvable fluorescent proteins paired with an oncogenic mutation of choice (positions 1-3) which in our case, is the mutated IDH1^{R132H} gene. Each R132H allele is co-expressed with TagBFP (cyan), mTFP1 (yellow), or mKO (magenta). The construct is further optimized to provide a rich fluorescent protein palette (XFP) by the expression of a membrane-targeted and chemically inducible near-infrared fluorogen-activating peptide (Fap-Mars1) as a control.
- We may introduce more than one copy of the Crainbow cassette in the nucleus to multiply the number of colors or experiment with different approaches for color cellular tagging which will make cell tracing and lineage analysis tasks easier.
- We will then have to follow a multi-step process to capture the collective mobility of the brain cancer cells:
  - Brain mouse organoids will be made according to established protocols.
  - We will use an RNA protein detection assay such as the RNAScope Kits from ACD to identify mRNA targets in FFPE tissues sections from crainbow mice brain.
  - We will then follow a single-cell RNASeq protocol to verify that the cancer cells have the expected IDH1 mutation and to confirm the genetic profile of our cell population in our organoids which involves more steps:
    - Cell isolation (we can use a single-cell isolator for single cell capture)
    - Build of gene matrices (or expression matrix in single-cell RNASeq methodology)
    - We can use a prepackaged software solution like "single cell sequencing without the instrument" [2]

  Now with color coding as cell identification, we can track more easily cell migration of the mutated cells:
- Tissues will be appropriately stained
- Imaging will be performed using a confocal microscope equipped with lasers[3]
- Excitation laser and appropriate detector ranges will be used for multispectral acquisition of XFPs and fluorescent stains.
- We may need to find a microscope with built-in software to acquire time-lapses images.

We will then perform a displacement analysis using the following metrics

- The mean squared displacement which describes the displacement of cells as the average distance of the subpopulations of cells (identified by the same color) in a given time interval, τ:

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[1] The knock-in region comprises a 5' loxP site, a wildtype min-gene (exons 3-9 and 3'UTR), an SSV40 polyA signal, a Neo^R cassette flanked by Frt sites, and a 3' loxP site.The LoxFtNwCD vector will be injected into C57BL/6 ESS cells. The Neo^R cassette will then be removed using Cre-mediated recombination and the R132H allele will be knocked-in *in vivo*.

[2] https://www.parsebiosciences.com/technology

[3] If too much photobleaching or phototoxicity are observed, we will use a two-photon excitation microscope.

$$MSD = \frac{1}{N} \sum_{n=1}^{N} \quad |r^n(\tau) - r^n(0)| \text{ where}$$

N: total number of trajectories for all measured subpopulations
$r^n(0)$: starting position of the nth cell trajectory
$r^n(\tau)$: position of the nth cell after τ

- Instantaneous speed, η, at the ith step of a cell as the displacement:

$$\eta = |\mathbf{v}(t_i)| = \frac{r(t_i) - r(t_{i-1})}{t_i - t_{i-1}}$$

- The migration speed of a cell, V, is the mean over the entire length of the trajectory from i = 1 to the total number of steps L: $V = \frac{1}{L} \sum_{i=1}^{L} \eta_i$

- The velocity auto-correlation function (VACF) of the color tag ensemble average of cell trajectories:

$$VACF = <\mathbf{v}(0) \bullet \mathbf{v}(\tau)> = \frac{1}{N} \sum_{n=1}^{N} \quad v^{(n)}(0) \bullet v^{(n)}(\tau)$$

where $v^{(n)}(0)$ is the initial velocity of the nth cell and $v^{(n)}(\tau)$ is its velocity at time τ.

- Directionality is given by the ration between cross-product and dot product between position vectors at t_i: $\mathbf{r}(t_i)$, and t_{i-1}: $\mathbf{r}(t_{i-1})$:

$$\tan(\theta) = \frac{|\mathbf{r}(ti) \ x \ \mathbf{r}(ti-1)|}{|\mathbf{r}(ti) \bullet \mathbf{r}(ti-1)|}$$

With these metrics, we will create various plots and run analysis:
counts (%) vs. density, MSD vs. time (τ), V vs. time (τ), VACF vs. time (τ), angles distributions vs. time (τ)

We will check then:
1. if the diffusion of migrating cells is characterized by a power law (MSD ~ τ^α, $\alpha < 1$: diffusion is sub-diffusive, $1 < \alpha < 2$: diffusion is super-diffusive, $\alpha = 2$: diffusion is ballistic)
2. if there is correlation decay over time: VACF diminishes with increased τ if the cells velocities become uncorrelated due to interactions with the surrounding environment.
3. type of cell motion: log-normal, Brownian walk or Levy walk[4]. Any Brownian random walk model of cell migration will be characterized by a uniform distribution of turning angles, θ, between successive time samples.

Lastly, we may explore to model IDH1 mutant cell mobility using more sophisticated mathematical models like persistent random walk (PRW) model, or sigmoidal Gompertzian model. This additional quantification of cell motility could be reused to verify drug efficiency targeting IDH1 (see table 1) when model predictions for these treated cancer cells could be different from the non-treated cancer cells and help to measure efficiency of the therapies (for example: decrease of cell motility indicating less cell spreading).

[1] Bardella et al., 2016, Cancer Cell 30, 578–594 - October 10, 2016 - http://dx.doi.org/10.1016/j.ccell.2016.08.017
[2] Peter G. Boone - A cancer rainbow mouse for visualizing the functional genomics of oncogenic clonal expansion Nature communications - https://www.nature.com/articles/s41467-019-13330-y
[3] Weissman TA, Pan YA. 2015 Brainbow: new resources and emerging biological applications for multicolor genetic labeling and analysis. Genetics 199(2):293- 306.
[4] Mehrjardi et al. Cell Death and Disease (2020)11:998 https://doi.org/10.1038/s41419-020-03196-0 - Current biomarker-associated procedures of cancer modeling-a reference in the context of IDH1 mutant glioma
[5] Woodworth et al.: Building a lineage from single cells: genetic techniques for cell lineage tracking - DOI: 10.1038/nrg.2016.159

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[4] Cell motion has been identified as either log-normal or a Lévy walk depending on the tissue they migrated in: G.M. Fricke, K.A. Letendre, M.E. Moses, J.L. Cannon, Persistence and adaptation in immunity: T cells balance the extent and thoroughness of search, PLoS Comput. Biol. 12 (2016) 1–23, and T.H. Harris, E.J. Banigan, D.A. Christian, C. Konradt, E.D.T. Wojno, K. Norose, E.H. Wilson, B. John, W. Weninger, A.D. Luster, et al., Generalized Lévy walks and the role of chemokines in migration of effector CD8+ T cells, Nature 486 (2012) 545.

[6] Nousi et al. Biochemistry and Biophysics Reports 28 (2021) 101120 Single-cell tracking reveals super-spreading brain cancer cells with high persistence
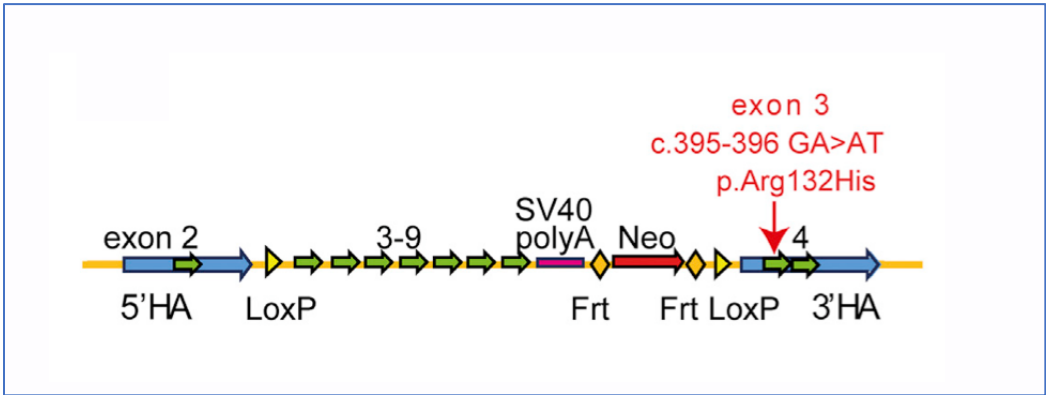
Construct used to generate IDH1-KI mice: loxP and Frt sites; 5' and 'homology arms; wild-type mini-gene (exons 3-9), SV40 polyA signal, neomycin resistance cassette (NEO[R]), and location of the R132H mutation.
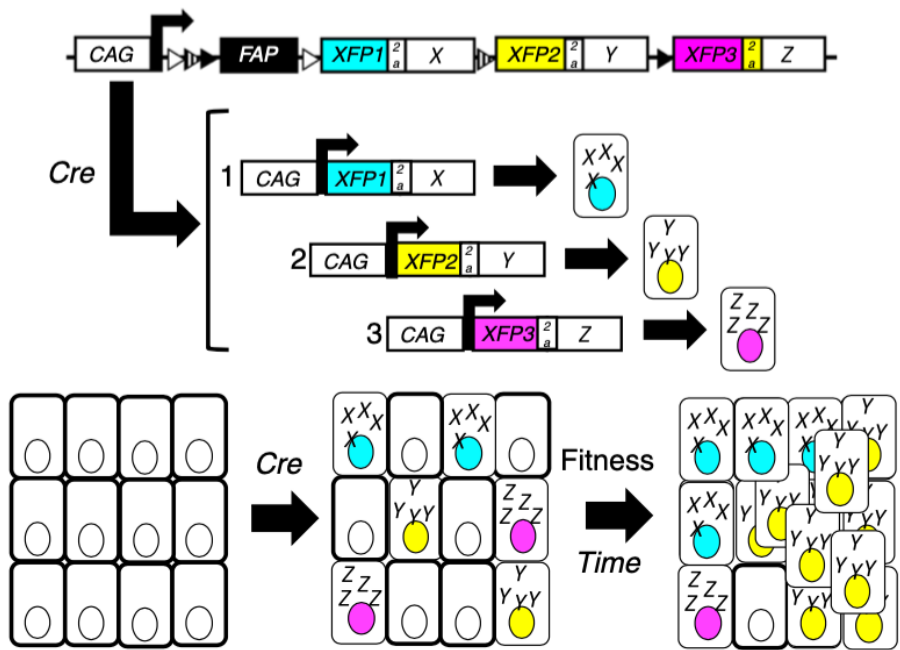
Stochastic Cre-mediated recombination and palette of colors for cell lineage
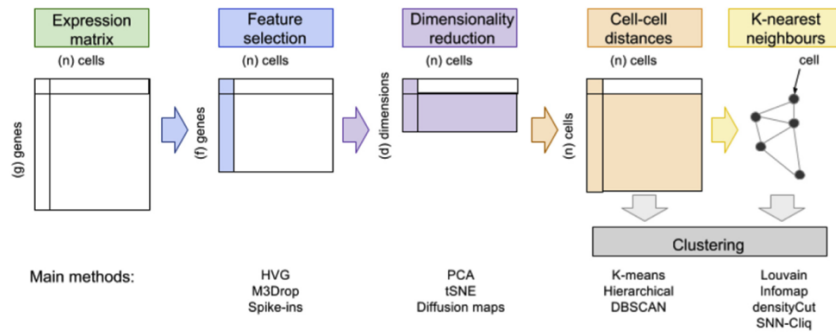
Overview of RNASeq protocol

Table 1 **Clinical trial of drugs or small molecules on gliomas containing the IDH1 mutation.**

Clinical trail.gov	Phase of trial	Compound/ treatment tool	Participants	Dosing	Result	Refs.
NCT02454634	I	IDH1 20 mer peptide vaccine (NOA-16)	39	8 times every 2/4 weeks	Cytotoxic immune response in and humoral immune responses	[79]
NCT02073994	I	AG-120	170	28-day cycles, oral	Ongoing trail	[80]
NCT02968940	II	Avelumab + Radiation	43	Every 2 weeks	–	[81]
NCT03212274	II	Olaparib	145	28-day cycles, oral	–	[81]
NCT02746081	I	BAY1436032	81	21-day cycles, oral	–	[82]
NCT02771301	I	IDH-R132H-DC vaccine	30	–	–	[83]
NCT02481154	I	AG-881 (ivosidenib)	95	28-day cycles, oral	Favorable safty at doses 100 mg and above	[84]
NCT03343197	II	AG-120 + AG-881	49	28-day cycles, oral	Ongoing trail	[85]
NCT02273739	I/III	AG-221	21	28-day cycles, oral	–	[86]
NCT02381886	I	IDH305	166	–	–	[87]
NCT02193347	I	IDH1 peptide vaccine	24	–	–	[88]
NCT03681028	I	AG-881		Oral	Tumor growth 6.8% compared to 24.5% placebo	[89]
NCT02209428	II	Temozolomide	54	Every 21 days, 6 cycles.	–	[90]
NCT03557359	II	Nivolumab	37	Every 2 weeks for 8 cycles for 2 years, infusion	Ongoing trail	[91]
NCT03960502	I	AG-881	5	Single dose, oral followed with infusion	–	[92]
NCT04164901	III	AG-881	366	Daily dosing, oral	Ongoing trail	[93]
NCT03684811	I/II	FT-2102 + Azacitidin	200	–	Ongoing trail	[94]
NCT03666559	II	Azacitidine	63	Seven consecutive days every 4 weeks, injection	–	[95]

Table 1