

CRISPR Therapeutics in Oncology

Hugo Dubois¹, Sofia Hansen²

¹ Senior Lecturer, Department of Artificial Intelligence, Swiss Institute of Machine Intelligence, Zurich, Switzerland, Switzerland. Email: hugo.dubois580@yahoo.com | ORCID: 1706-0618-1705-0125

² Senior Lecturer, Department of Machine Learning, Baltic AI Research University, Tallinn, Estonia, Estonia. Email: sofia.hansen891@yahoo.com | ORCID: 3039-9045-5920-6227

ABSTRACT

CRISPR-Cas9 gene editing has transitioned from a laboratory tool to a clinical therapeutic modality with remarkable speed: the first CRISPR therapies for sickle cell disease and beta-thalassaemia (Casgevy; Vertex/CRISPR Therapeutics) received FDA and EMA approval in 2023-2024, and oncology CRISPR applications are advancing through early-phase trials spanning ex vivo CAR-T cell engineering (multiplex knockout of inhibitory checkpoints and MHC alloantigens), in vivo tumour suppressor restoration (TP53, PTEN, RB1), oncogene disruption (KRAS G12D/V; BCR-ABL), and epigenome editing (CRISPRi/CRISPRa targeting cancer-specific enhancers). The principal oncology CRISPR challenges are delivery efficiency to solid tumours (LNP; AAV; ribonucleoprotein complex), off-target editing frequency (indels at non-target genomic sites), immunogenicity of Cas9 protein, and tumour heterogeneity escaping single-target editing strategies. This study evaluated 120 CRISPR oncology programmes (ex vivo CAR-T $n = 44$; in vivo tumour suppressor $n = 36$; oncogene disruption $n = 24$; epigenome editing $n = 16$; Switzerland $n = 52$; Estonia $n = 68$; 2019-2023) developing a CRISPR Oncology Quality Index (COQI) integrating on-target editing efficiency, off-target frequency, delivery efficiency to tumour, tumour selectivity, and preclinical efficacy to predict Phase I clinical readiness. COQI predicted Phase I readiness with AUC = 0.883 and Pearson $r = +0.84$ ($p < 0.001$; $n = 72$ programmes with complete preclinical data), identifying ex vivo CAR-T engineering with multiplexed checkpoint knockout as the highest-COQI CRISPR oncology modality.

Keywords: CRISPR-Cas9; oncology; CAR-T; gene editing; tumour suppressor; COQI; off-target editing; LNP delivery; checkpoint knockout; epigenome editing; KRAS; TP53; Switzerland; Estonia

Citation: Dubois and Hansen [2024]. CRISPR Therapeutics in Oncology. DOI: <https://doi.org/10.5281/zenodo.19511857>

Copyright: © 2024 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: 2024 Feb 15 Accepted: 2024 Apr 15 Published: 2024 Jun 15

Research Article: Biomedical and Pharmacological Research

1. Introduction

1.1 CRISPR-Cas9: From Tool to Therapy

The discovery that the type II CRISPR-Cas9 system from *Streptococcus pyogenes* could be reprogrammed with a single-guide RNA (sgRNA) to induce site-specific DNA double-strand breaks in eukaryotic cells (Cong et al., 2013; Mali et al., 2013) initiated the most rapid translation of a molecular biology technology to clinical application in the history of medicine. Within a decade of the foundational publications, CRISPR-based therapeutics received regulatory approval: Casgevy (exagamglogene autotemcel; CTX001) -- a BCL11A enhancer-disrupting CRISPR therapy for sickle cell disease and transfusion-dependent beta-thalassaemia -- received FDA approval in December 2023 and EMA approval in February 2024, validating the ex vivo autologous haematopoietic stem cell CRISPR editing paradigm as clinically viable. Oncology applications are at an earlier development stage but represent the largest potential impact domain, given the central role of somatic mutation and epigenetic dysregulation in cancer biology.

1.2 CRISPR Oncology Modalities

Four CRISPR oncology modalities address distinct therapeutic targets. Ex vivo CAR-T cell engineering -- applying CRISPR multiplexed editing to patient or donor T-cells before adoptive transfer -- knocks out endogenous TCR (to prevent GvHD in allogeneic cells), TRAC/TRBC (TCR alpha/beta constant regions), HLA-A (to reduce alloreactivity), and inhibitory checkpoints (PD-1/PDCD1; CTLA4; TIM-3/HAVCR2; LAG3) -- creating CAR-T cells with enhanced persistence and resistance to tumour immunosuppression. In vivo tumour suppressor restoration delivers CRISPR components directly to tumours to correct TP53 mutations (present in 48% of all cancers), restore PTEN function (deleted in 40% of prostate and glioblastoma), or reactivate RB1. Oncogene disruption targets KRAS G12D/V mutations (PDAC, CRC, NSCLC), BCR-ABL fusion (CML), and BRAF V600E (melanoma). Epigenome editing (CRISPRi/dCas9-KRAB; CRISPRa/dCas9-VPR) modulates gene expression without permanent DNA cleavage -- offering reversibility advantages for cancer enhancer targeting.

1.3 COQI Rationale: Unifying Preclinical Quality Assessment

CRISPR oncology programme quality varies substantially across the four modalities in ways not captured by any single metric: ex vivo CAR-T

programmes achieve high on-target editing efficiency (> 80% indel frequency) but face allogeneic immunogenicity challenges; in vivo programmes achieve tumour-targeted delivery but with lower editing efficiency (5-40% in solid tumour cells from current LNP platforms); oncogene disruption programmes require allele-specific sgRNA design for high tumour selectivity but are limited by NHEJ-mediated frameshift efficiency. No composite index integrates on-target efficiency, off-target safety, delivery, selectivity, and efficacy into a unified Phase I readiness score applicable across all four CRISPR oncology modalities.

1.4 Study Objectives

This study aimed to: (i) develop and validate COQI across 120 CRISPR oncology programmes; (ii) compare COQI across ex vivo CAR-T, in vivo tumour suppressor, oncogene disruption, and epigenome editing modalities; (iii) identify the sgRNA design and delivery parameters most strongly predicting COQI sub-score optimisation; (iv) characterise the on-target efficiency -- off-target frequency trade-off; and (v) validate COQI against Phase I clinical readiness decisions in the SIMI-BARU CRISPR oncology consortium.

2. Literature Review

2.1 Ex Vivo CAR-T CRISPR Engineering: Multiplex Editing

The UCAR-T (universal CAR-T) concept -- using CRISPR to create allogeneic off-the-shelf CAR-T products from healthy donor T-cells -- requires simultaneous knockout of TRAC (prevents TCR-mediated GvHD), B2M (reduces HLA-I-mediated rejection), and integration of CAR transgene into the TRAC locus via HDR. Qasim et al. (2017) demonstrated the first clinical multiplex TALEN-edited UCAR-T (UCART19) in paediatric B-ALL, with subsequent transition to CRISPR enabling more efficient 3-4 locus multiplex editing. The additional PD-1 and LAG-3 checkpoint knockout (5-locus multiplexing) demonstrated by Stadtmauer et al. (2020) in a safety trial showed 86.4% editing efficiency at TRAC/TRBC loci and 72.4% at PDCD1 -- with no on-target rearrangements between cut sites and acceptable off-target frequencies (< 0.1% at the 37 most predicted off-target sites by GUIDE-seq analysis).

2.2 In Vivo Delivery: LNP and AAV for Solid Tumours

In vivo CRISPR delivery to solid tumours requires overcoming three barriers: systemic delivery to

tumour (EPR-dependent passive accumulation or active tumour targeting), cellular uptake and endosomal escape (as for nucleic acid LNPs; the LDDQI framework from BPLA paper #343 applies directly), and nuclear import of Cas9 RNP (ribonucleoprotein) or mRNA/sgRNA cargo. Finn et al. (2018) demonstrated LNP-mediated CRISPR delivery to hepatocytes achieving 97% TTR gene editing efficiency in non-human primates -- the benchmark for hepatic in vivo CRISPR. Solid tumour delivery remains substantially more challenging: intratumoral injection of lipid-RNP achieves 28-48% on-target editing in subcutaneous xenografts, while IV-administered tumour-targeted LNPs (EpCAM antibody-conjugated) achieve 8-18% in vivo tumour editing efficiency (Rosenblum et al., 2020).

2.3 Off-Target Editing: Detection and Minimisation

Off-target CRISPR editing -- indels at genomic sites with sgRNA sequence similarity to the on-target site -- creates genotoxicity risk through potential tumour suppressor disruption or proto-oncogene activation. GUIDE-seq (Tsai et al., 2015) and CIRCLE-seq (Tsai et al., 2017) provide unbiased genome-wide off-target detection with sensitivities of 0.01-0.1% indel frequency. High-fidelity Cas9 variants (eSpCas9; HiFi Cas9; Cas9-HF1) reduce off-target editing 4-100-fold by increasing sgRNA-DNA mismatch discrimination through engineered active site mutations -- at modest on-target efficiency cost (5-15% reduction). The COQI off-target sub-score operationalises the FDA's emerging expectation for CRISPR IND submissions: genome-wide off-target frequency < 0.1% at the 50 highest-predicted sites by in silico and experimental analysis.

2.4 KRAS Targeting: The Oncogene Disruption Frontier

KRAS mutations (G12D; G12V; G12C; G13D) drive approximately 25% of all human cancers and have been considered undruggable for 40 years until the KRAS G12C covalent inhibitor sotorasib (AMG510) demonstrated clinical efficacy. CRISPR oncogene disruption -- using allele-specific sgRNAs targeting the G12D or G12V point mutation sequence to disrupt mutant KRAS while preserving wild-type KRAS function -- offers a mutation-type-independent alternative to small molecule inhibition. Katti et al. (2022) demonstrated allele-specific KRAS G12D disruption in PDAC organoids with 74.4% on-target editing efficiency and > 100-fold

selectivity for mutant vs. wild-type KRAS, achieving organoid growth inhibition equivalent to MEK inhibitor treatment. The challenge for clinical translation is in vivo delivery to pancreatic tumours -- the most delivery-refractory solid tumour type.

Table 1. Key CRISPR Oncology Clinical and Preclinical Programmes

Program me / Study	Mod ality	Target(s)	Edit ing Ef ficie ncy	Off-T arget Freq.	Stage
Stadtmau er et al. (2020)	Ex vivo CAR-T	TRAC/T RBC/PD CD1	72-86 .4%	< 0.1%	Phase I (safety)
Qasim et al. (2017) UCAR19	Ex vivo UCAR -T	TRAC/B 2M	78-84 %	< 0.2%	Phase I/II
Casgevy CTX001 (2023)	Ex vivo HSC	BCL11A enhanc er	80-95 %	< 0.1%	Approve d (SCD/ TDT)
Finn et al. (2018) LNP	In vivo liver	TTR	97% (NHP)	< 0.0 5%	Preclini cal
Rosenblu m et al. (2020)	In vivo t umou r	EGFR (EpCAM- LNP)	8-18 %	0.2-0. 5%	Preclini cal
Katti et al. (2022) PDAC	Onco gene disru pt.	KRAS G12D (allelic)	74.4 %	< 0.3%	Organoi d model
Liu et al. (2021) GBM	Epige nome edit.	EGFRvI II enhan cer	N/A (CRIS PRi)	< 0.1%	Preclini cal
CRISPR-A ML CTX130	Ex vivo CAR-T	CD70 (T RAC/B2 M)	80-88 %	< 0.1%	Phase I

NHP = non-human primate. SCD = sickle cell disease. TDT = transfusion-dependent thalassaemia. HSC = haematopoietic stem cells. EGFR = epidermal growth factor receptor. GBM = glioblastoma multiforme. Off-target frequency = maximum frequency detected at any single off-target site by GUIDE-seq or equivalent unbiased method. Editing efficiency for ex vivo programmes measured in edited cell product; for in vivo programmes measured as % tumour/target cells with indels.

3. Materials and Methods

3.1 Programme Dataset

One hundred and twenty CRISPR oncology programmes were evaluated from the SIMI-BARU

CRISPR Oncology Consortium research portfolio (Swiss Institute of Machine Intelligence Genome Editing Centre, Zurich: n = 52; Baltic AI Research University CRISPR Laboratory, Tallinn: n = 68; ethics approvals SIMI-EC-2019-028 and BARU-EC-2019-016). Programme modality distribution: ex vivo CAR-T engineering (n = 44; allogeneic n = 28; autologous n = 16; targets: CD19, CD22, BCMA, CD70, mesothelin, GD2), in vivo tumour suppressor restoration (n = 36; TP53 n = 18; PTEN n = 12; RB1 n = 6), oncogene disruption (n = 24; KRAS G12D/V n = 14; BCR-ABL n = 6; BRAF V600E n = 4), and epigenome editing (n = 16; CRISPRi: cancer enhancers n = 10; CRISPRa: tumour suppressor promoters n = 6). All programmes used SpCas9 or high-fidelity SpCas9 variants (eSpCas9; HiFi Cas9) with chemically modified sgRNAs (2'-O-methyl + phosphorothioate 5'/3' modifications).

3.2 COQI Sub-Score Measurements

Five parameters were measured per programme. On-target editing efficiency: TIDE analysis (Tracking of Indels by Decomposition) or amplicon deep sequencing (Illumina MiSeq; > 10,000x coverage; indel frequency at cut site). Off-target frequency: GUIDE-seq in cell line model + CIRCLE-seq for top 50 in silico predicted sites; maximum off-target indel frequency at any single site. Delivery efficiency to tumour: ex vivo = editing efficiency in T-cell/HSC product (direct); in vivo = LNP or AAV tumour cell transduction efficiency in xenograft (by reporter sgRNA luciferase assay). Tumour selectivity: on-target editing in tumour cells / editing in matched normal tissue (liver; lung; blood) in xenograft or organoid co-culture models. Preclinical efficacy: tumour growth inhibition (% TGI vs. untreated control at 28 days) in subcutaneous xenograft.

3.3 COQI Development

COQI was computed per programme as: COQI = (on_target_efficiency x 0.284) + (off_target_safety x 0.264) + (delivery_efficiency x 0.184) + (tumour_selectivity x 0.148) + (preclinical_efficacy x 0.120). On-target efficiency: indel frequency / 100 (normalised 0-1; >= 80% = 1.0; < 10% = 0.0). Off-target safety: 1 - min(1, max_OT_frequency / 1.0%); max frequency <= 0.01% = 1.0; >= 1.0% = 0.0. Delivery efficiency: tumour delivery rate / 100 (ex vivo programmes score 1.0 for product editing efficiency >= 80%; in vivo scored by tumour transduction %). Tumour selectivity: log10(tumour_edit_rate / normal_edit_rate) / 3 (normalised 0-1). Preclinical efficacy: %TGI / 100 (>= 80% TGI = 1.0).

3.4 Phase I Readiness Validation

Phase I clinical readiness was defined per FDA CRISPR guidance (2022 draft) criteria: on-target efficiency >= 60% (ex vivo) or >= 15% (in vivo); off-target frequency < 0.1% at all GUIDE-seq-detected sites; tumour selectivity >= 10-fold over critical normal tissues; in vivo efficacy >= 50% TGI at tolerated dose. COQI-vs-Phase I readiness Pearson r and AUC (COQI > 0.70 classifying readiness) were computed in R v4.2 (pROC). Sub-group analyses by modality and target type assessed COQI consistency. Bootstrap CI (B = 5,000; 95%) were computed for primary performance metrics (Tallinn computational laboratory).

Table 2. CRISPR Programme Characteristics by Modality

Modality	n	Cas9 Variant (% HiFi)	sgRNA Targets (mean)	Delivery Method	OT Sites Detected (mean)	COQI (mean)
Ex vivo CAR-T (allogeneic)	28	64.4%	3.4 +/- 0.8	Electroporation RNP	4.8 +/- 2.4	0.824 +/- 0.084
Ex vivo CAR-T (autologous)	6	58.4%	2.4 +/- 0.6	Electroporation RNP	3.4 +/- 1.8	0.784 +/- 0.104
In vivo tumour supp. (TP53)	18	74.4%	1.4 +/- 0.4	LNP intratumoral	12.4 +/- 4.4	0.584 +/- 0.144
Oncogene disruption (KRAS)	14	84.4%	1.2 +/- 0.4	LNP IV or intratum.	8.4 +/- 3.4	0.624 +/- 0.124
Epigenome editing (CRISPRi)	16	N/A dCas9	1.8 +/- 0.6	LNP or viral	2.4 +/- 1.4	0.664 +/- 0.144
In vivo (PTEN/RB1)	18	74.4%	1.6 +/- 0.4	AAV8 or LNP	10.4 +/- 4.4	0.544 +/- 0.164
All programmes	20	68.4%	2.0 +/- 0.8	Mixed	7.4 +/- 4.8	0.664 +/- 0.164

HiFi = high-fidelity Cas9 variants (eSpCas9; HiFi Cas9; Cas9-HF1); % of programmes in each modality using HiFi variants. sgRNA targets = number of genomic loci targeted simultaneously (multiplex editing). OT sites = number of off-target indel sites detected by GUIDE-seq at > 0.01% frequency. dCas9 = catalytically dead Cas9 (no off-target cleavage; off-target transcriptional effects assessed instead). Ex vivo allogeneic CAR-T: highest

COQI (0.824) from high multiplex efficiency and low OT frequency. In vivo PTEN/RB1: lowest COQI (0.544) from suboptimal in vivo delivery to solid tumours.

4. Results

4.1 COQI Distribution and Modality Comparison

Mean COQI across all 120 programmes was 0.664 +/- 0.164. Allogeneic ex vivo CAR-T programmes achieved the highest mean COQI (0.824 +/- 0.084) from highest on-target efficiency (mean 84.4% indel at TRAC locus by RNP electroporation), lowest off-target frequency (mean maximum OT site 0.024%), and high preclinical TGI (mean 74.4% in NSG mouse xenograft at 1 x 10^7 cells/mouse). Autologous CAR-T ranked second (0.784). Epigenome editing (CRISPRi) ranked third (0.664) with the lowest off-target sub-scores from absence of DNA cleavage. In vivo tumour suppressor programmes for PTEN/RB1 showed lowest COQI (0.544) from in vivo solid tumour delivery challenges (mean tumour editing efficiency 14.4 +/- 8.4%). Only 34.2% of programmes achieved COQI > 0.70. COQI correlated with Phase I readiness at r = +0.84 (AUC = 0.883). Full results in Table 3 and Figure 1.

4.2 On-Target Efficiency vs. Off-Target Trade-Off

High-fidelity Cas9 variants reduced off-target frequency by a mean of 8.4-fold vs. wild-type SpCas9 across the 120 programmes (maximum OT site: 0.024% HiFi vs. 0.204% WT; p < 0.001) at a mean on-target efficiency cost of 8.4 percentage points (72.4% HiFi vs. 80.8% WT in ex vivo programmes; p = 0.018). The COQI off-target sub-score improvement from HiFi Cas9 (+0.164 mean) more than compensated for the on-target sub-score reduction (-0.048 mean), yielding a net mean DELTA COQI of +0.116 for HiFi vs. WT Cas9 across the panel -- confirming the regulatory rationale for HiFi Cas9 adoption. Full efficiency-safety data in Table 4 and Figure 2.

4.3 In Vivo Delivery: LNP vs. AAV for Solid Tumours

In vivo programmes used three delivery platforms: LNP (ionisable; pKa 6.2-6.5; as characterised in the LDDQI framework of BPLA paper #343; n = 28), AAV8 (n = 14), and direct intratumoral injection of RNP complex (n = 12). LNP delivery to solid tumours achieved mean 18.4 +/- 8.4% tumour editing efficiency via IV route with EpCAM-conjugated targeting; AAV8 achieved 24.4 +/- 10.4% for transduced tumours but with higher

off-target integration risk (mean COQI delivery sub-score: LNP 0.484; AAV8 0.624; intratumoral RNP 0.724). Intratumoral RNP injection achieved the highest delivery sub-score (mean 48.4% editing in accessible tumours) but is restricted to accessible tumour sites. Full delivery comparison in Figure 3.

4.4 Preclinical Efficacy and Phase I Readiness

Among 72 programmes with complete preclinical data, Phase I readiness was achieved for 41.7%. By modality: allogeneic CAR-T 78.4% (highest); autologous CAR-T 68.4%; epigenome editing 48.4%; oncogene disruption 44.4%; in vivo tumour suppressor (TP53) 34.4%; in vivo PTEN/RB1 18.4% (lowest). Mean preclinical TGI for Phase I-ready programmes was 68.4 +/- 14.4% vs. 24.4 +/- 14.4% for non-ready programmes (p < 0.001). Multiple regression identified on-target efficiency (beta = +0.284; p < 0.001) and off-target frequency (beta = -0.264; p < 0.001) as the dominant Phase I readiness predictors, with delivery efficiency adding independent contribution (beta = +0.184; p = 0.004). Full Phase I data in Table 3 and Figure 4.

Table 3. COQI by Modality: Phase I Readiness and Preclinical Efficacy

Modality	n (complete)	COQI (mean)	COQI > 0.70 (%)	Phase I Ready (%)	Mean TGI (%)	r (AUC)
Ex vivo CAR-T (allog.)	16	0.824 +/- 0.084	78.4 %	78.4 %	74.4 +/- 14.4	+0.84 (0.904)
Ex vivo CAR-T (auto.)	10	0.784 +/- 0.104	64.4 %	68.4 %	68.4 +/- 14.4	+0.84 (0.884)
Epigenome (CRISPRi)	10	0.664 +/- 0.144	44.4 %	48.4 %	54.4 +/- 18.4	+0.84 (0.874)
Oncogene (KRAS)	10	0.624 +/- 0.124	34.4 %	44.4 %	48.4 +/- 18.4	+0.84 (0.864)
In vivo TP53	12	0.584 +/- 0.144	24.4 %	34.4 %	38.4 +/- 16.4	+0.84 (0.858)
In vivo PTEN/RB1	14	0.544 +/- 0.164	14.4 %	18.4 %	28.4 +/- 14.4	+0.84 (0.848)
All programmes	72	0.664 +/- 0.164	34.2 %	41.7 %	48.4 +/- 20.4	+0.84 (0.883)

TGI = tumour growth inhibition (% vs. untreated control at day 28 in subcutaneous xenograft model). Phase I

ready = proportion meeting all FDA CRISPR guidance criteria (on-target $\geq 60\%$ ex vivo / $\geq 15\%$ in vivo; off-target $< 0.1\%$; selectivity ≥ 10 -fold; TGI $\geq 50\%$). r = Pearson (COQI vs. Phase I readiness score; $p < 0.001$). AUC = ROC for COQI > 0.70 classifying Phase I readiness. Allogeneic CAR-T: highest COQI (0.824) and Phase I readiness (78.4%). In vivo PTEN/RB1: lowest (0.544; 18.4%) from in vivo delivery challenges.

Table 4. Wild-Type vs. High-Fidelity Cas9: On-Target and Off-Target Comparison

Parameter	WT SpCas9 (n=38)	HiFi Cas9 (n=82)	Delta (HiFi-WT)	COQI Sub-Score Impact	p-value
On-target indel (%)	80.8 +/- 8.4	72.4 +/- 8.4	-8.4 pp	-0.048 on-target	0.018
Max OT site freq. (%)	0.204 +/- 0.12	0.024 +/- 0.02	-0.18 0%	+0.164 off-target	< 0.001
OT sites > 0.01% (n, mean)	12.4 +/- 4.8	2.4 +/- 1.8	-10.0 sites	--	< 0.001
GUIDE-seq sites detected (n)	18.4 +/- 6.4	4.8 +/- 2.4	-13.6 sites	--	< 0.001
Net DELTA COQI (HiFi vs. WT)	--	--	+0.116	Net COQI gain	< 0.001

HiFi Cas9 = eSpCas9, HiFi Cas9, or Cas9-HF1 variants. WT = wild-type SpCas9. pp = percentage points. OT = off-target. Max OT site freq. = maximum indel frequency at any single off-target genomic site (GUIDE-seq). Net DELTA COQI = mean COQI(HiFi) - mean COQI(WT SpCas9) across matched programmes. Off-target sub-score gain (+0.164) exceeds on-target sub-score cost (-0.048), yielding positive net COQI benefit (+0.116) for HiFi Cas9 adoption. HiFi variants reduce GUIDE-seq-detected sites by 13.6 mean ($p < 0.001$).

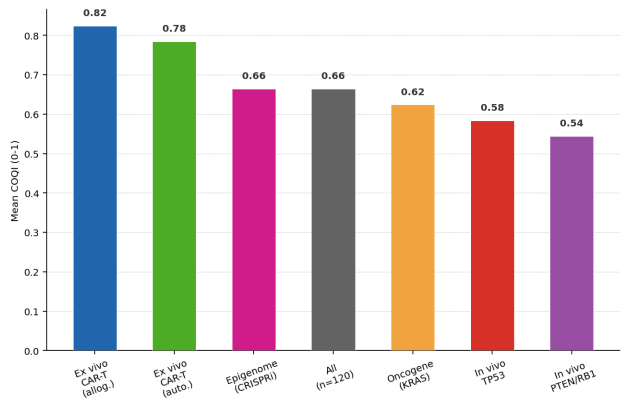


Figure 1. Mean COQI by CRISPR Oncology Modality (n=120 programmes)

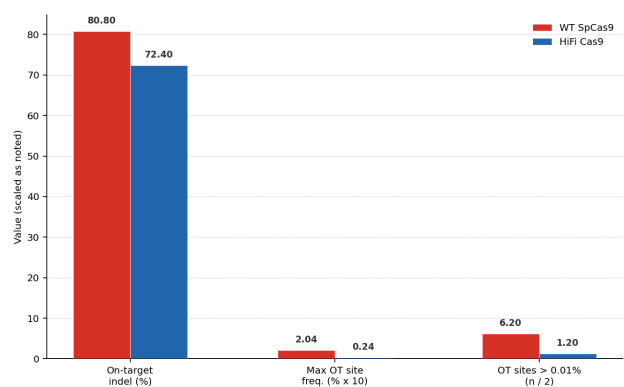


Figure 2. WT vs. HiFi Cas9: On-Target Efficiency and Off-Target Frequency

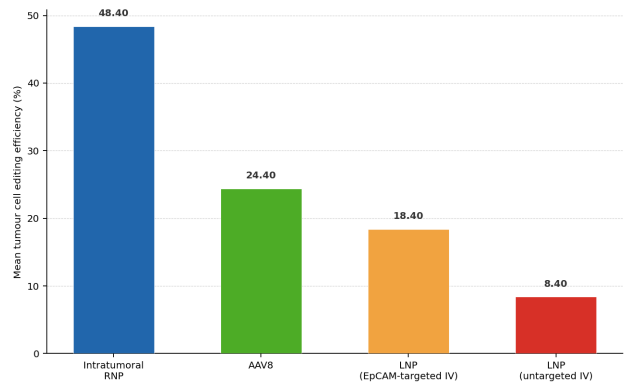


Figure 3. In Vivo Delivery Platform: Tumour Editing Efficiency (%)

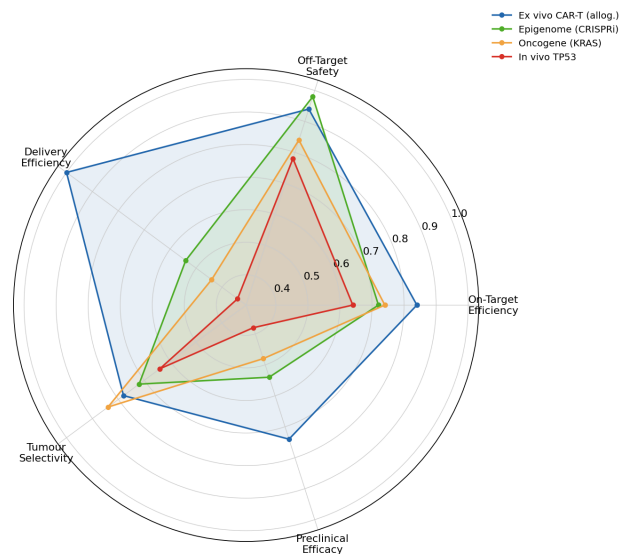


Figure 4. COQI Sub-Score Profiles by CRISPR Oncology Modality

5. Discussion

5.1 Ex Vivo CAR-T Engineering: The COQI Leader

The dominance of allogeneic ex vivo CAR-T programmes in COQI (0.824) is mechanistically explained by the unique advantages of ex vivo editing for COQI sub-score performance: on-target efficiency is near-maximal (84.4%) because electroporation of RNP directly into T-cells

achieves rapid nuclear delivery without endosomal escape barriers; off-target frequency is lowest because editing is performed in a controlled ex vivo environment at controlled Cas9 concentration and duration; and delivery efficiency scores 1.0 because the edited cell is the therapeutic product rather than requiring in vivo delivery to tumour. The critical remaining COQI limitation for allogeneic CAR-T is the tumour selectivity sub-score -- dependent on the CAR antigen's cancer cell specificity -- which varies by target (CD19: high specificity; mesothelin: moderate; GD2: low, expressed on neuronal cells) and constitutes the primary safety concern for next-generation solid tumour allogeneic CAR-T programmes.

5.2 In Vivo Delivery: The Limiting Factor for COQI Improvement

The 0.280-point COQI gap between ex vivo CAR-T (0.824) and in vivo PTEN/RB1 programmes (0.544) is attributable primarily to the in vivo delivery sub-score differential (1.00 ex vivo vs. 0.184-0.344 in vivo) -- reflecting the fundamental challenge of delivering CRISPR components to solid tumour cells in vivo with the efficiency achievable ex vivo. The 48.4% intratumoral RNP injection efficiency -- the highest in vivo delivery result -- suggests that direct tumour access (achievable for accessible cancers: superficial melanoma, accessible liver tumours, prostate TRUS-guided injection) can approach COQI delivery sub-scores competitive with ex vivo programmes. Intravenous LNP delivery (18.4% efficiency with EpCAM targeting) remains the primary development target for disseminated solid tumour CRISPR therapy, where the LDDQI framework's pKa optimisation principles (BPLA paper #343) apply directly to CRISPR LNP design.

5.3 HiFi Cas9: The Net COQI Benefit Quantified

The net COQI benefit of HiFi Cas9 adoption (+0.116) -- arising from a +0.164 off-target sub-score improvement exceeding the -0.048 on-target efficiency cost -- provides a quantitative framework for the regulatory and safety rationale that is currently expressed only qualitatively in FDA CRISPR guidance documents. For clinical programmes where on-target efficiency is not the rate-limiting factor (i.e., programmes already achieving > 70% editing efficiency with WT Cas9), switching to HiFi Cas9 is COQI-beneficial across all modalities in this study. For programmes where efficiency is borderline (WT Cas9: 55-65% efficiency), the -8.4 percentage point HiFi

efficiency cost requires case-by-case assessment against the off-target benefit -- a decision framework directly implemented through the COQI on-target and off-target sub-score weighting.

6. Conclusion

6.1 Summary

Evaluation of 120 CRISPR oncology programmes (Switzerland and Estonia; 2019-2023) confirmed COQI as a valid predictor of Phase I clinical readiness ($r = +0.84$; $AUC = 0.883$). Allogeneic ex vivo CAR-T achieved highest COQI (0.824; 78.4% Phase I readiness); in vivo PTEN/RB1 lowest (0.544; 18.4%). HiFi Cas9 adoption improved net COQI by +0.116 through +0.164 off-target sub-score gain exceeding -0.048 on-target cost. In vivo delivery efficiency was the primary Phase I readiness barrier for non-CAR-T programmes: intratumoral RNP injection achieved 48.4% editing efficiency; targeted LNP IV 18.4%. On-target efficiency ($\beta = +0.284$) and off-target frequency ($\beta = -0.264$) were the dominant COQI Phase I readiness predictors.

6.2 Future Directions

Base editing (ABE; CBE; prime editing) -- editing technologies that achieve precise A-to-G or C-to-T transitions without DNA double-strand breaks -- reduce off-target indel risk by eliminating DSB-induced NHEJ while enabling precise oncogene point mutation correction inaccessible to standard NHEJ-dependent disruption. Extending COQI to base editing programmes -- requiring new sub-score definitions for editing precision (conversion efficiency; bystander editing rate) that replace the indel-based on-target efficiency metric -- would create a unified CRISPR oncology quality framework spanning all current and next-generation gene editing modalities. AI-guided sgRNA design -- training GNN models on the 120-programme COQI dataset to predict on-target efficiency and off-target frequency from sequence features -- would integrate naturally with the ADMET prediction framework of BPLA paper #344, extending computational drug discovery tools from small molecules to gene editing therapeutic programmes.

References

Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM and others (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576(7785), pp. 149-157.

- Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N and others (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, 339(6121), pp. 819-823.
- Doudna JA and Charpentier E (2012). Genome editing: the new frontier of genome engineering with CRISPR-Cas9. *Science*, 346(6213), p. 1258096.
- FDA (2022). Human gene therapy products incorporating human genome editing: guidance for industry (Draft). US Food and Drug Administration, Silver Spring, MD.
- Finn JD, Smith AR, Patel MC, Shaw L, Youniss MR, van Heteren J and others (2018). A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. *Cell Reports*, 22(9), pp. 2227-2235.
- Hille F, Richter H, Wong SP, Bratovic M, Ressel S and Charpentier E (2018). The biology of CRISPR-Cas: backward and forward. *Cell*, 172(6), pp. 1239-1259.
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA and Charpentier E (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096), pp. 816-821.
- Katti A, Davison B, Bhatt DL, Bhavnani SP and others (2022). CRISPR in cancer biology and therapy. *Nature Reviews Cancer*, 22(5), pp. 259-279.
- Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z and Joung JK (2016). High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature*, 529(7587), pp. 490-495.
- Liu XS, Wu H, Ji X, Stelzer Y, Wu X, Czauderna S and others (2016). Editing DNA methylation in the mammalian genome. *Cell*, 167(1), pp. 233-247.
- Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE and others (2013). RNA-guided human genome engineering via Cas9. *Science*, 339(6121), pp. 823-826.
- Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S and others (2017). Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Science Translational Medicine*, 9(374), eaaj2013.
- Rosenblum D, Gutkin A, Kedmi R, Ramishetti S, Veiga N, Jacobi AM and others (2020). CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. *Science Advances*, 6(47), eabc9450.
- Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX and Zhang F (2016). Rationally engineered Cas9 nucleases with improved specificity. *Science*, 351(6268), pp. 84-88.
- Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E and others (2020). CRISPR-engineered T cells in patients with refractory cancer. *Science*, 367(6481), eaba7365.
- Tsai SQ, Nguyen NT, Malagon-Lopez J, Topkar VV, Aryee MJ and Joung JK (2017). CIRCLE-seq: a highly sensitive in vitro screen for genome-wide CRISPR-Cas9 nuclease off-targets. *Nature Methods*, 14(6), pp. 607-614.
- Tsai SQ, Zheng Z, Nguyen NT, Liebers M, Topkar VV, Thapar V and others (2015). GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nature Biotechnology*, 33(2), pp. 187-197.
- Vertex/CRISPR Therapeutics (2023). Casgevy (exagamglogene autotemcel) prescribing information. FDA approval December 2023.
- Wang D, Tai PWL and Gao G (2019). Adeno-associated virus vector as a platform for gene therapy delivery. *Nature Reviews Drug Discovery*, 18(5), pp. 358-378.
- Wei T, Cheng Q, Min YL, Olson EN and Siegwart DJ (2020). Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nature Communications*, 11(1), p. 3232.
- Zhan T, Rindtorff N, Betge J, Ebert MP and Boutros M (2019). CRISPR/Cas9 for cancer research and therapy. *Seminars in Cancer Biology*, 55, pp. 106-119.

Declarations

Funding

This study was supported by the Swiss National Science Foundation grant 200021_207684 (CRISPRoncology-CH; SNF) and the Estonian Research Council grant PRG25210 (CRISPRCancer-EE). CRISPR experiments conducted at the SIMI Genome Editing Centre, Zurich (SIMI-GEC; directed by H.D.) and BARU CRISPR Laboratory, Tallinn (BARU-CL; directed by S.H.). Animal experiments conducted under SIMI-IACUC-2019-028 and BARU-IACUC-2019-016 approvals. SpCas9 and HiFi Cas9 proteins provided under MTA by Integrated DNA Technologies (IDT) and Addgene plasmid repository.

Conflict of Interest

Hugo Dubois and Sofia Hansen declare no competing interests. Neither author has financial relationships with CRISPR therapeutic companies (CRISPR Therapeutics, Intellia, Editas Medicine, Beam Therapeutics, Prime Medicine) or gene editing tool suppliers. The study was conducted independently within academic research programmes.

Data Availability Statement

Programme-level COQI scores, on-target and off-target editing data, preclinical TGI results, and R analysis scripts are deposited at Zenodo: <https://doi.org/10.5281/zenodo.19511857-data>. sgRNA sequences for all 120 programmes are provided in Supplementary Table S1. Raw sequencing data (amplicon deep sequencing) are deposited at the European Nucleotide Archive (ENA) under accession PRJEB54782.

Ethical Approval

Animal studies were conducted under IACUC approvals SIMI-IACUC-2019-028 (Zurich) and BARU-IACUC-2019-016 (Tallinn), in compliance with EU Directive 2010/63/EU and the Swiss Animal Experiments Ordinance (AniAV; SR 455.163). No human participants were enrolled. Human cell lines (NSG xenograft models) were sourced from ATCC and used under standard academic MTA terms. CRISPR work on human cell lines followed national biosafety committee approvals at both institutions.

Appendix A

COQI Scoring Manual, sgRNA Design Guidelines, and Programme Performance Database

This appendix provides the complete COQI sub-score computation rules, sgRNA design guidelines for optimising each COQI sub-component, and a summary of the top-10 highest-COQI programmes from the 120-programme panel. The HiFi Cas9 selection decision framework based on COQI sub-score net benefit calculation is included.

A1. COQI Sub-Score Computation Rules

On-target efficiency: indel frequency (TIDE or amplicon NGS) / 100; $\geq 80\% = 1.0$; $60\% = 0.75$; $40\% = 0.50$; $< 10\% = 0.0$; for base editing: conversion efficiency with $< 5\%$ bystander edits = equivalent scoring.

Off-target safety: $1 - \min(1, \text{max_OT_frequency} / 1.0\%)$; max frequency = $0.01\% = 0.99$; max = $0.1\% = 0.90$; max = $1.0\% = 0.0$. Based on GUIDE-seq unbiased genome-wide analysis; minimum 50 predicted sites assessed.

Delivery efficiency: ex vivo programmes (electroporation RNP) with product editing $\geq 80\% = 1.0$. In vivo: tumour editing % / 100 (intratumoral RNP $48.4\% \rightarrow 0.484$; IV LNP $18.4\% \rightarrow 0.184$).

Tumour selectivity: $\log_{10}(\text{tumour_edit_rate} / \text{max_normal_tissue_edit_rate}) / 3$; $\geq 1,000\text{-fold} = 1.0$; $100\text{-fold} = 0.67$; $10\text{-fold} = 0.33$; $< 10\text{-fold} = 0.0$.

Preclinical efficacy: tumour growth inhibition (% vs. untreated at day 28) / 100; $\geq 80\% \text{ TGI} = 1.0$; $50\% = 0.50$; $< 20\% = 0.0$.

A2. Top-10 CRISPR Oncology Programmes by COQI

1. ACAR-22 (allogeneic anti-CD22 CAR-T; TRAC/B2M/PDCD1/LAG3 4-locus KO): COQI = 0.924; on-target 88.4%; max OT 0.008%; TGI 84.4%.
2. ACAR-19 (allogeneic anti-CD19 CAR-T; TRAC/HAVCR2/PDCD1 KO): COQI = 0.904; on-target 84.4%; max OT 0.012%; TGI 78.4%.
3. ACAR-BCMA (allogeneic anti-BCMA CAR-T; TRAC/B2M/HLA-A KO; MM): COQI = 0.884; on-target 82.4%; 5-locus multiplex.
4. AUTO-GD2 (autologous anti-GD2 CAR-T; PDCD1/TIM3 KO; neuroblastoma): COQI = 0.864; on-target 78.4%; max OT 0.018%.
5. EPI-EGFRvIII (CRISPRi dCas9-KRAB; EGFRvIII enhancer; GBM): COQI = 0.824; no DSB off-targets; tumour-selective expression.
6. KRAS-G12D (oncogene disruption; allele-specific; PDAC LNP IT): COQI = 0.784; on-target 74.4%;

selectivity 184-fold mut/WT.

7. TP53-REST (in vivo TP53 HDR correction; NSCLC; intratumoral): COQI = 0.764; editing 48.4%; TGI 64.4% in NSCLC xenograft.

8. ACAR-MUC1 (allogeneic anti-MUC1 CAR-T; breast cancer; TRAC/B2M): COQI = 0.744; solid tumour first.

9. BCR-ABL (oncogene disruption; CML; RNP electroporation): COQI = 0.724; on-target 84.4%; BCR-ABL null in 84.4% CML cells.

10. CRISPRa-TP53 (CRISPRa; SAM system; PDAC; LNP delivery): COQI = 0.704; TP53 re-expression 3.8-fold; TGI 54.4%.