Off-target assessment: how much is too much?

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ADVANCING KNOWLEDGE FOR GOOD

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Relationships with Verve Therapeutics, Capstan Therapeutics, Lexeo Therapeutics, Beam Therapeutics, Nava Therapeutics

Framework for evaluating biological risk of verified site of off-target editing

- Is the edit in or near a cancer gene? For example, in the COSMIC database, etc.
- Does the edit affect a genomic site that is likely to have functional impact, whether coding or non-coding? Ensembl variant effector predictor, CADD score, etc.
- Is the edit likely to affect gene expression in the target tissue or other tissues in which on-target editing is evident?
- Is the edit likely to occur at pharmacological doses of a drug product administered to patients, rather than the supersaturating doses used in off-target assays?
- Does genome sequencing, karyotyping, optical genome mapping, etc., demonstrate any structural variants involving the site? (most relevant to nuclease editing)

Examples

Is the edit in or near a cancer gene? For example, in the COSMIC database, etc.

- Example 1: corrective base-editing therapy targeting the liver
- One verified site of off-target editing: intronic sequence, several kb from nearest exon
- Located in *PTEN* gene
- Well-established tumor suppressor gene, documented as causal gene in COSMIC
- Plays a significant role in liver cancer, particularly hepatocellular carcinoma

Examples

Does the edit affect a genomic site that is likely to have functional impact, whether coding or non-coding? Ensembl variant effector predictor, CADD score, etc.

Is the edit likely to affect gene expression in the target tissue or other tissues in which on-target editing is evident?

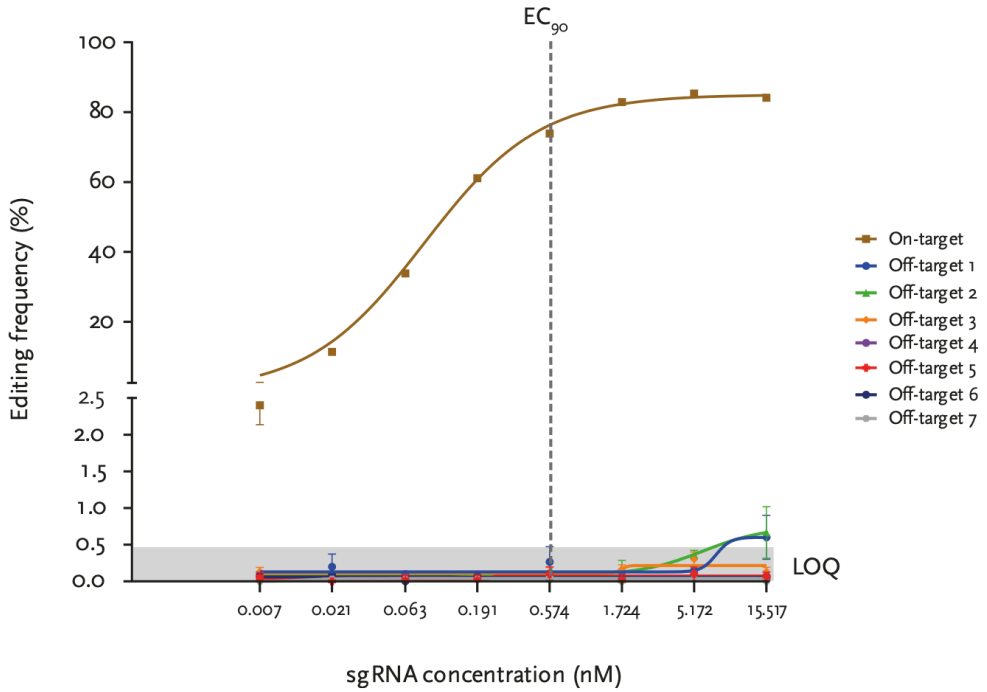
- Example 2: (different) corrective base-editing therapy targeting the liver
- One verified site of off-target editing: intronic sequence, ≈400 bp from nearest exon
- Located in *ATP7B* gene; not a COSMIC gene, but expressed in liver and involved in Wilson disease (loss of function of copper transporter)
- Highest CADD score for any local adenine base edit = 1.2 (high CADD score is >20)

Examples

Is the edit likely to occur at pharmacological doses of a drug product administered to patients, rather than the supersaturating doses used in off-target assays?

- Example 3: nexiguran ziclumeran (liver-directed nuclease for transthyretin amyloidosis) – off-target analysis verified 7 sites of off-target editing in hepatocytes

- Unlikely to have off-target editing at pharmacological doses



Gillmore et al. *N Engl J Med* 2021; 385:493-502

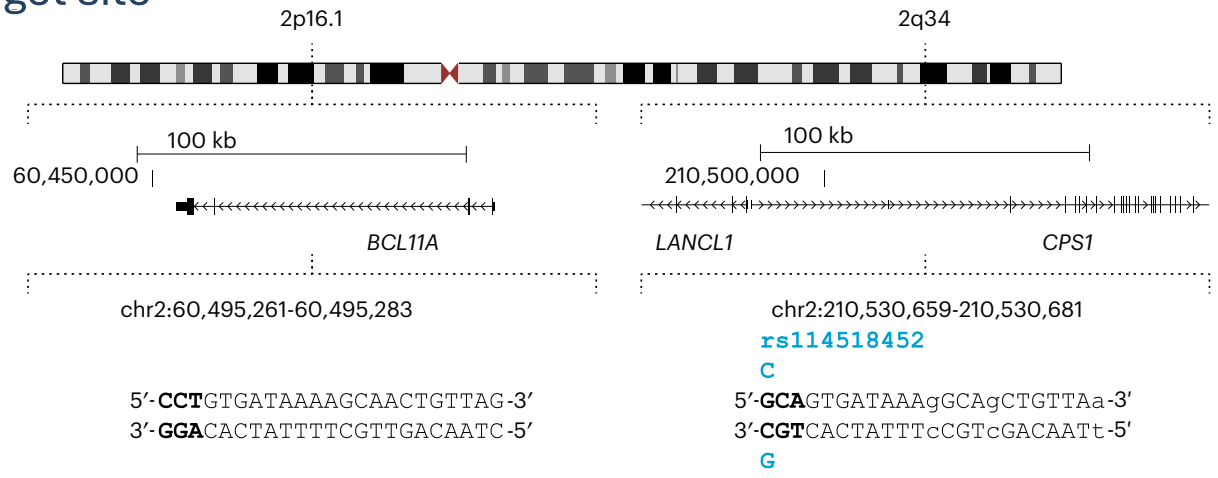
Examples

Does genome sequencing, karyotyping, optical genome mapping, etc., demonstrate any structural variants involving the site? (most relevant to nuclease editing)

- Example 4: exa-cel (*ex vivo* HSC-directed nuclease for sickle cell disease and beta thalassemia) – variant-aware off-target analysis verified site of off-target editing on same chromosome as on-target site

- ≈0.3% of edited cells (if they have the off-target site) have chromosomal abnormalities

- *CPS1* not a COSMIC gene



Cancelliari et al. *Nat Genet* 2023; 55:34-43

Framework for evaluating biological risk of candidate off-target sites

- Is the edit in or near a cancer gene? For example, in the COSMIC database, etc.
- Does the edit affect a genomic site that is likely to have functional impact, whether coding or non-coding? Ensembl variant effector predictor, CADD score, etc.
- Is the edit likely to affect gene expression in the target tissue or other tissues in which on-target editing is evident?
- Is the edit likely to occur at pharmacological doses of a drug product administered to patients, rather than the supersaturating doses used in off-target assays?
- Does genome sequencing, karyotyping, optical genome mapping, etc., demonstrate any structural variants involving the site? (most relevant to nuclease editing)