Alix VENTURES

Market Deep Dive Report

CRISPR Therapeutics

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Contents

1. Summary

Since its initial discovery as a genetic reprogramming tool in 2012, CRISPR has widely been regarded as a breakthrough scientific discovery and quickly become one of the hottest new biotechnologies in the industry. However, the CRISPR therapeutics market is still in early stages, with leading therapies only just reaching human clinical trials. Furthermore, the industry is highly defended by IP, and currently addresses a narrow set of indications. Top companies including Beam Therapeutics, Editas Medicine, and Prime Medicine have been founded by the same scientists, and have formed a highly collaborative moat. Remaining companies have partnered with large pharmaceutical companies to fund operations.

The future of CRISPR therapeutics development is dependent on technological advances in delivery methods and editing safety and granularity. Establishing that the agent can be delivered in an animal model in a manner that reaches the target tissue, understanding to the extent possible any potential side-effects and how to best manage them, optimizing editing efficiency in the target tissue in animals, developing practical ways to manufacture the candidate therapeutic at the high quality and consistency level required for use in humans, and several other key requirements are also crucial to developing new therapies. These are expensive and time consuming processes that are not well suited for teams without leading scientific expertise and many years of funding runway.

Overall, the CRISPR therapeutics market has started to saturate, with low hanging indications dominated by leading scientists and their companies. IP is also owned and collaboratively licensed among industry leaders, adding to the difficulty of market entry. New opportunities in the field are highly competitive and expensive to enter, with few seed or pre-seed rounds and large ($30 M+) series A rounds typical. This report finds few opportunities for CRISPR powered therapeutics for a Seed stage fund.

2. Market Overview

The global CRISPR and Cas gene therapeutics market was worth $1.4 billion in 2017 and is projected to grow at a CAGR of 20.8% through 2026 to reach $7.6 billion. This growth will be driven by increased R&D spending as well as the clinical maturation of existing pipelines. CRISPR development towards therapeutics started roughly in 2013, and only recently has the technology progressed such that patients have enrolled in small pilot tests. As clinical data

continues to mature and be released, the expectation is that it will facilitate increasing funding interest.

The industry is led primarily by small cap companies including Beam Therapeutics, Editas Medicine, CRISPR Therapeutics, and Intellia Therapeutics founded by scientific leaders including Feng Zhang, Jennifer Doudna, George Church, Keith Joung, and David Liu. Large biopharma companies including AstraZeneca, Pfizer, Regeneron, Novartis, and Vertex have engaged in partnerships to help develop their technology in exchange for IP rights, but most R&D activity is led by these smaller companies and their core scientists. The industry is heavily supported by academic development, specifically in the labs of the scientists who have co-founded the top companies in the field.

Historically, the market landscape has been differentiated by indication, but recently has trended towards technology based differentiation. Early market leaders namely Editas, Intellia, and CRISPR Therapeutics, in general are all applying the same CRISPR/Cas9 technology to perform genome editing, but have specialized towards specific indications, largely driven via partnerships and research collaborations. More recent startups have found other market niches driven by new technological developments such as CRISPR screening (KSQ Tx, REPARE Tx, Tango), base editing (Beam Tx), and prime editing (Prime Medicine).

2.1 Industry Challenges

As a gene therapy, there has been public controversy over the safety profile, ethics, and pricing of CRISPR therapeutics. For early stage startups in particular, there are another set of challenges stemming from the competitive nature of the field. Described below are the major hurdles to be aware of

1. Pricing will be an issue for patients and may come under scrutiny as more gene therapies are approved and start to put more pressure on the healthcare system. There has also been ethical debate, mostly stemming from germline editing, that has spilled over to somatic cell gene editing. Particularly due to high pricing, the ethics of only the wealthy being able to afford genetic cures has been an area of debate among bioethicists. Though this is unlikely to affect R&D, pricing regulations are an area where lawmakers have recently taken an interest and CRISPR may be disproportionately affected.

2. The safety profile of CRISPR therapeutics has not been fully validated. For in-vivo applications, robust data demonstrating lack of immunogenicity has yet to be published. For ex-vivo applications, researchers will need to demonstrate lack of immune rejection from transfused cells. Cell transfusions such as CAR-T also are frequently accompanied by poor side effects such as cytokine release syndrome or nausea from pre-treatment chemotherapy. Also important to consider are potential off-target edits by CRISPR machinery. Especially given the

history of gene therapy treatments, regulators have paid special attention to the safety profile of CRISPR and other gene therapies.

3. For startups, CRISPR therapeutics is a highly competitive and defended field to enter. Fundraising is plentiful, but CRISPR therapeutics have very long time tables, so many years of runway will need to be raised. Market leaders are founded by the scientific discoverers of CRISPR, meaning that a huge scientific and IP advantage is had by those companies. Due to the relative youth of CRISPR as a therapeutic strategy, there are few indications that have been targeted and are thus highly competitive.

2.2 Intellectual Property

Patents filed by UC Berkeley (Jennifer Doudna) and the Broad Institute (Feng Zhang) were filed in 2012 and 2014 for using CRISPR in bacterial strains and mammalian cells respectively. The dispute over whether these are distinct is ongoing and recently in 2019 was reopened by the Patent Trial and Appeal Board[1]. This has strong financial implications for industry leaders Beam, Intellia, Editas, and CRIPSR Therapeutics, whose scientific founders are the inventors of the 2012 patents. The Universities of California and the Broad Institute own the most extensive CRISPR patent portfolios. However, China has overtaken the U.S. in speed and volume since 2018.

The bottom line for startups is that the competitive nature of the field and the tremendou academic and commercial interests in the technology will make the IP landscape difficult to navigate. Licensing agreements will inevitably become a pain point as startups scale up and begin to commercialize their technology.

2.3 Regulatory Requirements

As of November 2017, the FDA considers CRISPR/Cas9 gene editing to be classified as gene therapies. Gene therapy products are regulated by the FDA's Center for Biologics Evaluation and Research (CBER). Clinical studies of gene therapy in humans require the submission of an investigational new drug application (IND) prior to their initiation in the United States, and marketing of a gene therapy product requires submission and approval of a biologics license application (BLA). Any trial involving the use of gene editing technology on humans must be individually approved by the FDA. Additionally, non-somatic germline editing is strictly prohibited.

The FDA has approved several gene therapy products including:

- Imlygic ~ a modified herpes virus used to infect melanoma cells and kill them.
- Kymriah ~ a CAR-T therapy: T-cells genetically modified to kill lymphoma and leukemia cancer cells

[1] Updates from Broad Institute

- Yescarta ~ a CAR-T therapy: T-cells genetically modified to kill lymphoma cancer cells
- Provenge ~ immune cells (APC) modified with PAP-GM-CSF to hunt down prostate cancer cells
- Luxturna ~ an AAV vector delivers RPE65 gene into the retina of patients with rare eye disease
- Zolgensma ~an AAV9 gene therapy delivers SMN1 gene to the motor neurons of SMA patients

Gene editing technologies including CRISPR will inevitably come under more intense scrutiny from regulatory agencies. However, given the classification as a gene therapy and the approval of various other gene therapy products, efficacy demonstrating CRISPR products should not be barred from market entry.

2.4 Pitchbook Market Statistics

- Quick stats (All time)
    - No. Companies: 78
    - No. Deals: 82
    - No. Investors: 101
    - Largest deal: $125 M (Beam Tx)
- Deal count (TTM): 24
- Most active VCs by deal count: ARCH Venture Partners, GV, F-Prime, Artis

Stage	**Average Round Size**	**Average Post Valuation**
Seed	$7 M	$23.7 M
A	$32.4 M	$82.3 M
B	$48 M	$255.75 M

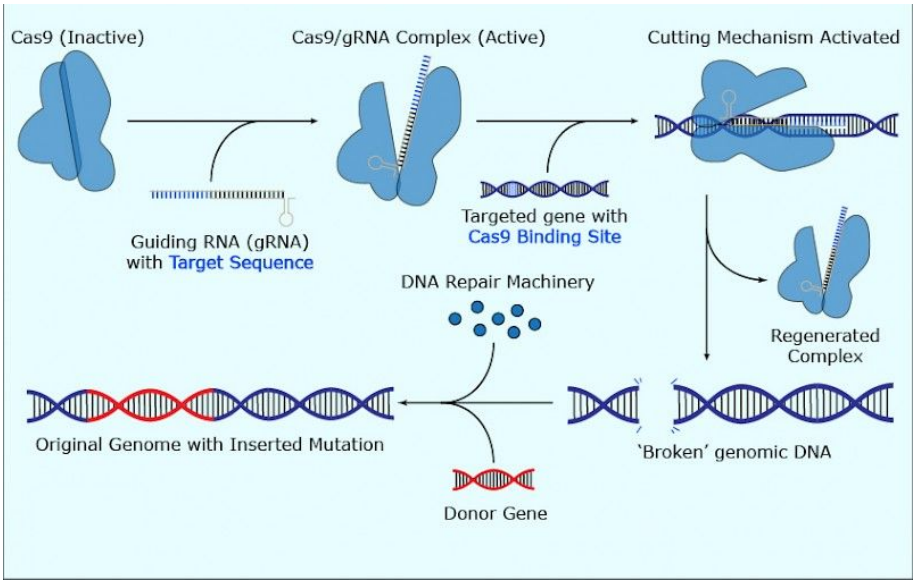
3. Technology Overview

3.1 Basic Summary

CRISPR/Cas gene editing technology arose from a bacterial defence mechanism against viral infections. It involves a guide RNA specific to a certain nucleic acid sequence, that leads a Cas protein to the site at which it binds. Binding sites must be accompanied by protospacer adjacent motifs (PAMs), which are extra base pairs in the DNA that the Cas protein needs to recognize in order to cut. Once the guide RNA binds to the host cell DNA, the Cas protein cuts

the strands apart, allowing free nucleotides or other local DNA sequences to fill in the gap via endogenous DNA repair mechanisms. The guide RNA can be synthesized specifically to bind to any sequence, thus allowing scientists to directly target certain genes or genetic defects.

Therapeutically, CRISPR can be used to knock out, repair, or insert genes into the genome of the host cell. Gene knockouts are the easiest task, as only the basic CRISPR machinery is required. The guide RNA will lead the Cas protein to the location to induce a break in the DNA, where afterwards random nucleotides will fill in the gap and deform the resulting proteins that are formed during translation. This random filling in of nucleotides relies on an endogenous repair mechanism called non-homologous end joining (NHEJ), which is highly efficient but introduces insertions or deletions that can shift the entire strand. A separate endogenous mechanism known as homology-directed repair (HDR) is far less efficient and also generates unwanted insertions or deletions, but has the potential to also insert larger strands of DNA if readily available. Ongoing CRISPR development is aimed at increasing the efficiency and frequency of HDR as a 'paste' gene editing function.



Simplification of CRISPR Process

Described above are all techniques that rely upon double stranded breaks to DNA. Gene or regulatory sequence disruption, or moving large segments of DNA, are well-suited to genome editing strategies that proceed through double-strand breaks. However, for precise point mutations where a single letter must be changed base or prime editing techniques have become the standard. Base editing was first described in 2016 from David Liu's lab at Harvard. Prime editing was just recently discovered also in David Liu's lab in late 2019.

3.2 Base Editing

Current base editors can offer higher editing efficiency and fewer indel byproducts (random insertions or deletions) than prime editors, while prime editors offer more targeting flexibility and greater editing precision. When the desired edit is a transition point mutation (C to T, T to C, A to G, or G to A), and the target base is well-positioned for base editing (that is, a PAM sequence exists approximately 15 bases from the target site), then base editing can result in higher editing efficiencies and fewer byproducts. Base editors have two principal components that are fused together to form a single protein: 1) a CRISPR protein, bound to a guide RNA, that leverages the established DNA-targeting ability of CRISPR, but modified such that they do not cause a double-stranded break, and 2) a base editing enzyme, such as a deaminase, which carries out the desired chemical modification of the target DNA base. This proprietary combination enables the precise targeting and editing of a single base pair of DNA.

By changing the guide RNA portion of the CRISPR protein, base editors can be targeted to different genomic locations based on their gene sequences. By changing the deaminase, the base that is edited (e.g., C or A) can be controlled. Base editors have several advantages over existing gene editing approaches: 1) The creation of precise, predictable and efficient genetic outcomes at a targeted sequence 2) High efficiency editing without need for template-based homology directed repair, and 3) Avoidance of the unwanted consequences of double-stranded DNA breaks, such as frequent insertions and deletions or larger-scale genomic rearrangements.

3.3 Prime Editing

For classes of mutations other than the four types of point mutations that base editors can make (C to T, T to C, A to G, or G to A), such as insertions, deletions, and the eight other kinds of point mutations, prime editing is currently the only approach that can make these mutations in human cells without requiring double-stranded DNA cuts or separate DNA templates. When the target base is not well-positioned for base editing, or when other "bystander" C or A bases are nearby that must not be edited, then prime editing offers major advantages since it does not require a precisely positioned PAM sequence and is a true "search-and-replace" editing capability, with no possibility of unwanted bystander editing at neighboring bases.

Prime editing heavily modifies the Cas9 protein and the guide RNA. The altered Cas9 only "nicks" a single strand of the double helix, instead of cutting both. The new guide, called a pegRNA, contains an RNA template for a new DNA sequence, to be added to the genome at the target location. That requires a second protein, attached to Cas9: a reverse transcriptase enzyme, which can make a new DNA strand from the RNA template and insert it at the nicked site.

Summary of CRISPR Proteins	
Cas9 (iSpyMac)	Good at cutting, great for knockouts. Produces a double stranded break

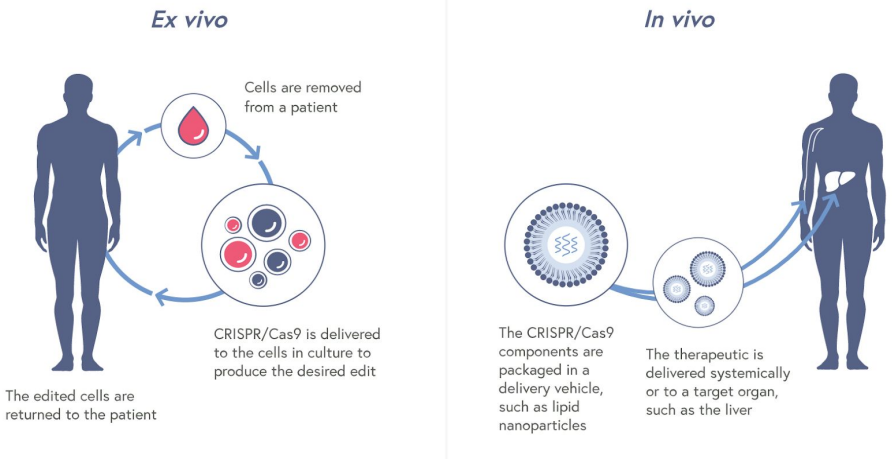
	that can be repaired via NHEJ or HDR
Cas12b	Cas12b is smaller than Cas9, which makes it promising from a delivery standpoint. However, it is harder to manufacture, has not been shown to work at mammalian temperatures, and is still an early stage research project.
Cas12a (Cpf1)	Cas12a has been used by Editas Medicine as a complementary tool to Cas9 that enables a broader range of possible edits.
Cas13	Cas13 is an RNA guided, RNA targeting protein that can be used to knock down protein translation without altering genome. Currently it has use in diagnostics, but use as a therapeutic is still in early research stages.
CasX/Y	These proteins were discovered in foreign bacteria and are roughly 40% smaller than Cas9, making them a promising option for improving delivery in AAV or other small vectors. These are in early research stages
Base Editing	Made up of a CRISPR protein and guide RNA plus a deaminase that makes a desired transition point mutation (C to T, T to C, A to G, or G to A). Invented by David Liu's lab and used by Beam Tx
Prime Editing	A research stage technology made up of an altered Cas9 that nicks the opposite DNA strand and a reverse transcriptase that synthesizes DNA from an RNA template and inserts it into the cellular DNA. Technology is licensed to Beam Tx and also invented by David Liu's lab.
CRISPRa and CRISPRi	CRISPR technology has also been used to activate or inactivate certain genes without editing DNA. CRISPR activation and inhibition (CRISPRa and CRISPRi) are promising due to the efficiency and accuracy of CRISPR machinery in binding to specific sequences. This is still in research stages

3.4 Ex-Vivo CRISPR

The most clinically advanced gene editing strategies rely on ex vivo cell manipulation that provides therapeutic effects following the administration of the cells back to the donor. In this way, CRISPR is used as a support technology for other therapeutics that require genetic manipulation of cells such as in adoptive transfer immunotherapy or stem cell therapies. CRISPR can be a preferred method of gene editing because of low costs and high efficiency, but they can also offer the technological advantage of replacement instead of co-expression. Other gene therapies such as those utilizing mRNA still allow the original unwanted gene to be expressed, but CRISPR cuts these out while replacing them with the wanted construct.

A promising example has been CAR-T therapies, where a patient's T-cells are genetically engineered ex-vivo to express a cancer targeting chimeric antigen receptor (CAR). In the case of CAR-T therapy support, they also provide a technological advantage, preventing overstimulation of T-cells or graft vs host complications that other viral mechanisms might induce. CRISPR techniques have also been useful for T-cell applications to knock out PD-1, a receptor that tumors target to stay invisible from immune surveillance.

Ex-vivo is clinically advanced in large part because the delivery of CRISPR machinery is far easier without the barriers provided by one's body. Immune clearance, degradation by enzymes, or poor tracking to the target organs/cells are no longer challenges in a dish. One of the common techniques for ex-vivo delivery has been electroporation, where an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing DNA or RNA to be introduced into the cell. In addition to electroporation, any of the in-vivo delivery methods described below, can also be used.



Ex-Vivo vs In-Vivo

3.5 In-Vivo CRISPR

Perhaps the biggest challenge for CRISPR systems has been in-vivo gene editing. One of the biggest risks is that viral delivery tools or genome editing parts will provoke dangerous immune reactions in a patient. Another challenge is making sure that CRISPR enzymes degrade fast enough after doing their job, since leftover enzymes would give them a greater chance of cutting in unwanted places in the DNA.

One of the major design considerations that impact the efficacy of CRISPR systems in-vivo is delivery. There needs to be a mechanism by which CRISPR machinery can enter cells in order to have an editing effect, and there needs to be specific targeting to desired cell types. dTypically, delivery of CRISPR is done by packaging the mRNA encoding CRISPR machinery

(the Cas protein) into delivery vectors, the most common of which are lipid nanoparticles (LPs) and adeno associated virus (AAV) vectors.

With AAVs, DNA encoding CRISPR machinery is loaded into the virus, which then infects cells, thereby causing them to produce the necessary gene editing tools. AAV is not known to cause or relate with any diseases in humans. There is also a wide range of known AAV strains which allow for infection of a multitude of cells with different specificities. The virus itself is able to efficiently infect cells while provoking little to no innate or adaptive immune response or associated toxicity However, Cas9 and guide RNA are roughly 4.2kB in size, and the overall size of AAV (~20 nm) only allows for ~4.5–5 kb of genomic material. Thus, AAVs have been somewhat limited by cargo size. However, more compact Cas proteins are under development. This will be especially important for base and prime editing technologies. Base editors plus a guide RNA and associated regulatory sequences are around 6,000 base pairs. And prime editors plus a guide RNA and associated regulatory sequences are around 7,000 base pairs.

LPs deliver CRISPR machinery by encapsulating DNA or mRNA in a lipid coating and releasing it into the cytoplasm once the particle is taken up by the cells. Lipid nanoparticles do not contain any viral components, which helps minimize safety and immunogenicity concerns. There are some difficulties with efficiency, as LPs will sometimes get degraded by cellular exosomes. However, they continue to be developed, and increasingly have been engineered to carry more cargo, have higher transfection efficiency, and lower off target effects. CRISPR Therapeutics licenses delivery technology from MIT for LPs and StrideBio for AAVs. Similarly, other major CRISPR companies have also taken the strategy of licensing technologies for CRISPR delivery.

3.6 Alternative techniques and platforms

Zinc Finger Nucleases (ZFNs): ZFNs are based upon natural human proteins that can recognize DNA sequences and create double stranded breaks. A single zinc finger domain can recognize a sequence of three nucleotides, so a series of them can be fused together to create a DNA binding protein. Each ZFN is made by fusing together a DNA binding protein and a DNA cleaving protein, which bind specifically and cut the DNA into separate strands. ZFNs have low immunogenicity since the proteins are human derived, and since the proteins are relatively small, they are easy to deliver. However, because the proteins must be engineered to bind specifically to a given DNA sequence, they are in practice more expensive and difficult to manufacture. Additionally, unlike CRISPR, which has a generalized Cas nuclease that can be led by several different guide RNAs, the guide protein for ZFNs is fused to the DNA cleaving protein, meaning that it is more difficult to edit multiple points at once (multiplexing). ZFN technology is highly proprietary and is owned by Sangamo Therapeutics, making it difficult for startups to enter the space without licensing IP.

Transcription Activator-Like Effector Nucleases (TALENs): TALENs similarly to ZFNs are restriction enzymes that can be engineered to cut specific sequences of DNA. Transcription activator-like effectors (TALEs) can be engineered to bind to practically any desired DNA sequence, so when combined with a nuclease, DNA can be cut at specific locations. Unlike ZFNs, each TALE recognizes a single nucleotide, instead of three at a time, which makes the manufacture of TALENs easier. However, because many more domains are needed per sequence, TALENs are difficult to deliver in-vivo. Cellectis is developing CAR-T therapies utilizing TALENs as a gene editing tool. Due to difficulties in in-vivo delivery, TALENs have mainly been used for ex-vivo gene editing platforms.

Peptide nucleic acids (PNAs): On the cutting edge are PNAs, which are being developed by Trucode, a startup that came out of stealth in 2019 with a $34 million funding round led by GV and Kleiner Perkins. PNAs are synthetic mimics of DNA that hybridize with complementary DNA/RNAs with high binding strength and resistance to enzyme degradation. PNAs also do not cause double-stranded breaks seen with CRISPR and other nuclease-based editing technologies. Early stage research at Yale demonstrated that PNAs loaded into biodegradable polymer nanoparticles induce gene-editing and reverse disease phenotype in multiple animal models of disease, including beta-thalassemia and cystic fibrosis.

All of these competing platforms each have complementary roles for different purposes alongside CRISPR. In total, 74% of gene editing programs use CRISPR/Cas9. However, ZFNs and TALENs are further along in clinical trials. There are three major advantages of CRISPR systems that have led to their widespread adoption and development.

First, CRISPR does not require protein engineering, which decreases manufacturing complexity and increases development speed. CRISPR has these advantages because it relies on nucleic acid base pairing, instead of protein to nucleic acid pairing. Proteins are difficult to manufacture specifically to bind to certain sequences, whereas a corresponding nucleic acid sequence can easily be synthesized to the exact sequence desired. The job specific components of a CRISPR system are cheap and easy to manufacture, while the Cas protein that breaks open the DNA does not need to be tailored to a specific target. Also because there are no expensive protein engineering steps, it is easier for CRISPR systems to edit a high number of cells at once, with multiple gene targets.

Second, there is diversity among Cas protein variants, which have allowed different molecular functions utilizing the same basic construct. This has widened CRISPR adoption and broadened its application to many other domains. Finally, the open access policy of the CRISPR research community has promoted widespread uptake, in contrast to the proprietary nature of ZFN platform.

3.7 Late stage privates & publics

Editas Medicine: Editas was founded in 2013 by giants in CRISPR editing including Feng Zhang, George Church, Jennifer Doudna, David Liu, and Keith Joung. After raising $210 million through series B, Editas IPOed in early 2016 and currently has a market cap of $1.5 billion. Editas has a diverse pipeline of both in-vivo programs (ocular and neurological) as well as cell engineering programs (hematology and cancer). Their furthest candidate, EDIT-101, treats Leber Congenital Amaurosis 10 and is currently in early stage human testing.

Intellia Therapeutics: Intellia was founded in 2014 and raised $85 million in two venture rounds before it IPOed in 2016. Its current market cap is $1.06 billion and its founding team includes Dr. Jennifer Doudna. Through partnerships with Regeneron and Novartis, Intellia is developing therapeutics for a variety of blood based indications both in-vivo and ex-vivo. Their furthest candidate (QTQ923) has reached early stage clinical trials and involves engineering of stem cells to treat sickle cell disease.

Beam Therapeutics: Beam was founded in 2018 and quickly raised $222 million in two venture funding rounds before their IPO in early 2020 (current market cap: $1.4 billion). Beam's scientific founders are Feng Zhang, Keith Joung, and David Liu and thus have close collaboration with Editas medicine, and David Liu's new startup, Prime Medicine. The indications it aims to tackle are broad, including the blood disorders sickle cell disease and beta thalassemia; T-cell acute lymphoblastic lymphoma and acute myeloid leukemia in oncology; liver diseases, including alpha-1 antitrypsin deficiency and glycogen storage disorder 1a; and Stargardt disease, an inherited form of macular degeneration, and other ocular and central nervous system disorders. The driving technology used by Beam is base editing, and the company plans an initial wave of submissions in 2021 requesting the FDA's OK to start clinical studies.

CRISPR Therapeutics: CRISPR Therapeutics was founded in 2014 and raised $127 million in three venture rounds behind Emmanuelle Charpentier and Matthew Porteus. The company IPOed in 2016 and has the largest market cap of its competitors at $3.82 billion. CRISPR Therapeutics has programs for hemoglobinopathies such as sickle cell and beta-thalassemia (collaboration with Vertex), allogeneic CAR-T gene editing, regenerative medicine (stem cell editing for diabetes), and in-vivo programs for Duchenne's Muscular Dystrophy, Cystic Fibrosis, and glycogen storage disease.

3.8 Key People and Labs

Feng Zhang: Dr. Feng Zhang spent his early career pioneering early work in optogenetics, but currently leads a lab at MIT studying in vivo modulation of biological function and delivery mechanisms. He is the founder of Beam Therapeutics, Editas Medicine, and Sherlock

Biosciences. He is the holder of the MIT/Broad Institute patent in 2014 that described CRISPR use in editing mammalian cells.

David Liu: Dr. David Liu's lab at Harvard invented base and prime editing and currently conducts research on DNA templated synthesis, protein evolution, and genetic engineering. Research from his lab will be key in the development of high fidelity base and prime editing technology that is used by Beam Therapeutics, Editas Medicine, and Prime Medicine where he is a founder.

Jennifer Doudna: Dr. Jennifer Doudna is a HHMI funded professor at UC Berkeley where she became the first to patent CRISPR for use in bacterial strains alongside Emmanuelle Charpentier. She is a founder of Editas Medicine and Intellia Therapeutics and leads a lab studying CRISPR and other gene editing technologies.

Keith Joung: Dr. Joung is a founder at Beam Therapeutics and Editas Medicine and leads a lab at Harvard studying genetic and epigenetic engineering techniques using CRISPR/Cas9, TALENs, and ZFNs. He pioneered the development of designer nucleases.

Emmanuelle Charpentier: Dr. Charpentier is a French scientist at the Max Planck Institute that first co-patented CRISPR for use in bacterial strains. She is a founder and board member of CRISPR Therapeutics and conducts fundamental research on pathogens and implications for human disease.

Matthew Porteus: Dr. Porteus is faculty at Stanford and a scientific founder at CRISPR Therapeutics. He was the first to show that engineered nucleases could be used to precisely modify human cells by homologous recombination, and runs a lab dedicated to advancing gene editing via homologous recombination techniques.

4. Therapeutic Landscape

4.1 Present day status

Clinical trials are underway in three major treatment areas: cancers, blood disorders, and eye disease. In addition, there are discovery stage programs targeting other genetic diseases, regenerative medicine uses, and as a treatment for viral infections such as HIV. All current CRISPR clinical trials are intended to edit the specific tissues without affecting sperm or eggs, meaning no DNA changes can be passed onto future generations.

Eye Disease

Eye diseases are common targets for in-vivo genetic engineering due to the eye being immune privileged and easily accessible for injections, making delivery easier. Indications that currently have pipeline programs include Staghardt's disease, Usher Syndrome, Autosomal Dominant Retinitis Pigmentosa 4, and Lever Congenital Amaurosis (LCA).

As one example, LCA is the most common cause of inherited childhood blindness. The most common variant, LCA10, is caused by a mutation in a photoreceptor gene and is the target for the first in-vivo CRISPR trial. Researchers from Editas Medicine and collaborators at Allergan will deliver the CRISPR machinery (guide RNA and Cas protein) in an AAV vector specific to photoreceptor cells. A single dose is injected directly into the patient's eye.

Immuno-Oncology

In cancer, CRISPR technology has been utilized to 'knock in or out' genes associated in the development of cancer, such as PD-1 from immune cells. In these treatments, immune cells will be harvested from blood and genetically engineered to exhibit favorable tumor fighting characteristics. In particular, CRISPR has been utilized heavily for developing CAR-T therapies for acute myeloid leukemia, solid tumors, multiple myeloma, acute lymphoblastic leukemia, and for CD19+ malignancies.

In CAR-T therapies, a patient's T-cells are genetically engineered ex-vivo to express a cancer targeting chimeric antigen receptor (CAR). In the case of CAR-T therapy support, they also provide a technological advantage, preventing overstimulation of T-cells or graft vs host complications that other viral mechanisms might induce. CRISPR techniques have also been useful for T-cell applications to knock out PD-1, a receptor that tumors target to stay invisible from immune surveillance.

Blood Diseases

Blood diseases have become a common target for CRISPR therapies because cells can be engineered ex-vivo and infused back into the patient, making delivery easier. Mutations that affect hemoglobin are also common targets for CRISPR editing. In beta thalassemia, patients do not make enough hemoglobin which leads to anemia and fatigue. In sickle cell disease, misshapen blood cells cause complications in blood flow. Current CRISPR clinical trials for blood disorders increase levels of fetal hemoglobin, which can take the place of defective adult hemoglobin.

The first step of this treatment is to harvest a patient's blood stem cells from their blood, then CRISPR will be used to engineer them to turn on fetal hemoglobin. Chemotherapy will be used to remove the patient's remaining defective stem cells, and billions of genome-edited stem cells are put back into the patient's body. Ideally these cells will create a new blood stem cell population in the bone marrow, which will make red blood cells with fetal hemoglobin.

Gene-edited blood stem cells can be delivered by IV, and ex vivo editing makes it easy to deliver genome editing tools to the target cells. Vertex and CRISPR Therapeutics have developed

Other Genetic Diseases

The indications that CRISPR is able to treat are expanding, due to improvements in our delivery capacity as well as improved understanding of the genetic basis of diseases. In particular, autosomal recessive, chronic, genetic diseases such as cystic fibrosis (CF), Huntington's and duchenne muscular dystrophy (DMD) are promising indications where initial in-vivo data has been positive. Loss of function diseases are promising for gene therapies because a correction of even a modest fraction of target cells is thought to offer benefits to patients.

Vertex has taken a lead in collaboration with CRISPR Tx to treat DMD, and CF. Liver diseases are also common targets due to the ease of targeting with lipid nanoparticle vectors. Glycogen Storage Disorder 1a is a particular disease that has attracted interest from CRISPR Tx and Beam Tx. Below is a list of other indications that industry leaders and startups have targeted:

- Hereditary angioedema (Intellia Tx; IND-enabling stage)
- Transthyretin amyloidosis (Intellia Tx with Regeneron, starting early stage clinical trials)
- Type 1 Diabetes (CRISPR Tx; allogeneic beta cell replacement therapy; with ViaCyte)
- Neurological Diseases (Editas Medicine with AskBio, and Beam Tx)
- Alpha1 - Antitrypsin Deficiency (Beam Tx)
- HIV (Excision Bio, non-human primate data demonstrating signs of efficacy)

4.2 Future Indications

In the near term, CRISPR can be applied to immediate tools that are currently using gene editing including knockout animal models, isogenic cell lines, CAR T cells, etc. Currently, clinical translation of CRISPR is in very early stages. The initial human studies that are set to finish in 2020 and 2021 will provide data on efficiency, toxicity, off target effects, and efficacy that is currently unknown. Over the next five years, we will see a maturing drug pipeline that will eventually reach late stage clinical trials in select monogenic diseases. Hematopoietic diseases, such as hemophilia and sickle cell anemia, will likely be the first target to circumvent delivery challenges. Applications in other monogenic diseases will start to emerge, but mostly using ex vivo delivery.

In the long term, the advance of in vivo delivery will enable CRISPR application in more complex genetic diseases, which include hard-to-target monogenic disorders and multigenic disorders. With increasing development of base and prime editing, CRISPR will theoretically be

able to address essentially all types of genetic disorders, including those that base editing and the basic CRISPR/Cas9 system are unable to treat, such as Progeria or Tay-Sachs Disease. The continued growth and development of CRISPR technologies will depend on improvements in delivery technology, multiplexing efficiency, and the prevention of off target effects.

4.3 Startups to Watch

eGenesis: eGenesis was founded by George Church and Luhan Yang in 2014 to bring to life CRISPR based xenotransplantation. The company has raised a total of $140 million in funding through Series B to grow human compatible organs inside of pigs. Pig genomes can contain up to several dozen copies of porcine endogenous retroviruses, or PERVs, which can't be eliminated by breeding alone. PERVs are known to be infectious in humans and are one of the contributing factors to the failure of past xenotransplantation efforts. Addressing the ever-growing need for transplantable organs, eGenesis is working on eliminating these retroviral genes from pig genomes using CRISPR, making organ transplantation from pigs to humans a safe option.

Inscripta: Inscripta was founded in 2015 by Andrew Garst, Ryan Gill, and Tanya Lipscom to develop the next generation of CRISPR nucleases. These improved enzymes boast such innovative features as differing PAM recognition sequences, cut efficiencies, reduced sizes, and differing enzyme kinetics. Inscripta boasts a high-throughput multiplexed genetic engineering platform that can synthesize enhanced bespoke enzymes for specific customer uses. So far, the company has raised $260 million through Series D.

NTrans Technologies: NTrans was founded in 2015 by Marco de Boer to develop CRISPR delivery technology. Their method, termed "iTOP" (induced transduction by osmocytosis and propanebetaine), causes the cell to uptake large fluid-filled vesicles, known as macropinosomes, which can contain CRISPR guide RNAs and Cas9. These vesicles release their contents inside cells, allowing CRISPR genome editing to occur.

Ligandal: Ligandal was founded in 2013 by Andre Watson and Christian Foster and has since raised $4.6 million through Series A to use ligands to deliver gene therapies to their target areas. Their new technology streamlines the in vivo delivery mechanisms for CRISPR, RNA, and other genetic tools.

Verve Therapeutics: Verve was founded in 2018 to develop gene editing therapies for cardiovascular diseases. Their goal is to develop gene-editing therapies to reduce the risk of coronary artery disease in adults. In order to achieve this, they are developing gene therapies, administered in a single dose, that would act as naturally protective gene variants. This would then confer lifelong protection from coronary heart disease in adults. The company has raised $121.5 million through Series A.

Excision Bio: Excision is a seed stage company that has raised $10 million to develop CRISPR-based therapies to cure viral infectious diseases and improve the lives of chronically ill patients. Their initial target is HIV, and is the first company to completely remove HIV from the genomes of animals.

Eligo Biosciences: Eligo has raised $27.4 million to develop a new class of targeted biotherapeutic agents to selectively intervene on the microbiome. Eligo is using proprietary methods in synthetic biology, protein and genome engineering to create Eligobiotics: genetic circuits packaged in phage-based delivery vectors used to diagnose, eradicate, or functionalize targeted microbial populations.

5. Conclusions

In conclusion, CRISPR therapeutics are a quickly maturing area that will drive significant patient impact in blood diseases, cancer, eye diseases, and other genetic diseases. Continued development of improved CRISPR delivery mechanisms that reduce off target effects and increase editing efficiency will catalyze the translation of gene editing into clinics within the next 10 years. However, industry leaders bolstered by the scientific inventors and patent owners of CRISPR technology have built up a commanding development lead. CRISPR therapeutics have demonstrated to be a vertical with long and expensive time horizons, with no candidates past Phase 2 clinical trials. As a result, venture rounds have been large, and there have been few early seed stage startups.

5.1 Vertical Strengths

- Rapid technological development will open up door to future opportunities
- Clearly defined patient populations reduces market risk
- Large scientific interest will allow startups to be supported by a robust and energized academic infrastructure
- Favorable acquisition and collaboration environment from both an IP and research perspective

5.2 Vertical Weaknesses

- Large deal sizes, expensive to take to market, competitive funding environment for VCs
- Off targets effects and ethical debates have stalled progress in clinical trials
- IP is consolidated by top scientists and their companies, a highly defensible moat
- Delivery technology (targeting, toxicity, efficiency) has not matured for in-vivo applications
- Technically ‘easy’ indications are highly competitive

5.3 Opportunity Cost of Capital

CRISPR startups in the therapeutic domain have raised large financing rounds and have been keen to IPO early to raise additional funds. While top companies have been able to reach high valuations, few started out at low enough valuations for a seed stage fund to have an impact. Due to the competitive funding environment for CRISPR based technologies, larger VCs, pharmaceutical companies, and others with larger powder kegs have dominated fundraising opportunities. With the presence of powerful competitors and the moats they have developed from their IP, smaller scale startups are risky when tackling an established indication. For a seed stage startup to be successful, they must have strong academic ties to help fuel research, a novel and feasible indication to tackle, and a proprietary technological advantage.

5.4 Investment Theses

1. **CRISPR Delivery Technology**: Cell type and organ level specificity and efficiency of delivery has become a major challenge. In order for CRISPR technologies to become cost effective and clinical feasible, they must be delivered with high efficiency and with minimal off target effects. Companies that are developing better delivery technologies will be able to address this growing demand as gene therapies like CRISPR continue to progress.

2. **Indication Specificity**: While CRISPR technology is a promising platform gene therapy technology, deep understanding of the addressed indication is essential to clinical success. Companies such as Verve Therapeutics and Excision Bio are able to address indications where market dominance can be established. In contrast, the scientific giants in the space and their companies (Beam Tx, Editas, CRISPR Therapeutics) have half a decade in progress treating the low hanging fruit. Companies that understand the clinical landscape and target diseases without large competitors have the greatest chance of clinical scaleup.

6. References

1. CRISPR Cas Review
2. Genetic Editing Targeted Therapy Review
3. David Liu Interview
4. Regulatory
5. CRISPR Trials 2019 Updates
6. Intellia Data
7. Wiley CRISPR Review
8. Synapse Review
9. Prime Editing; News Article
10. Base Editing; News Review