2021 CRISPR Year in Review

Science Circle
January 29th 2022

Stephen Gasior, Ph.D.
a.k.a. Stephen Xootfly
Scientist
Corteva Agrisciences
Researcher at Corteva some of whose research is presented here. Not representing the company’s positions.

Nothing should be construed as investment advice or company forward-looking statements.
2021 CRISPR Year in Review

Background
Target Genes and Modified Organisms
- Human Disease Cure, HIV, bacteria mods, plants

Origin Story
- transposon career change

Cautionary Tales
- Messing up chromosomes

Technology Advances
- Tissue delivery, Inversions, Plant efficiency, ONYX

New Tools
- Payloads, miniCas, COVID screen, reprogrammed
2021 CRISPR Year in Review

Cas9 First and most studied and used

Cas12a Second most well studied (large families of Cas12s)

Cas13a targets RNA

Technique allows us to localize a protein to precise locations via RNA homology

A double strand break can modify or add sequences at a target location.

2021 CRISPR Year in Review

Base Editing – one nucleotide change
Prime Editing
1) template sequence for the repair
2) small but precise insertions or deletions
No DSBs

**Target Genes and Modified Organisms**

“CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis”

**Single CRISPR injection to “cure” disease**

Transthyretin amyloidosis

- Buildup of mutant protein, expressed in liver but circulated in blood
- Polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, and gastrointestinal features
- Severe diarrhea with malabsorption, cachexia, incapacitating neuropathy, severe cardiac disturbances, and marked orthostatic hypotension
- Death usually occurs 5 to 15 years after onset of symptoms

Target Genes and Modified Organisms

“CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis”

Single CRISPR injection to “cure” disease

Fig. 2 Reductions from Baseline in Serum TTR Protein Concentration after Infusion of NTLA-2001 in Humans.


van Kampen, Sebastiaan Johannes, and Eva van Rooij. *Nature Biotechnology* 39.8 (2021): 920-921 (Review)

“Brain-wide Cas9-mediated cleavage of a gene causing familial Alzheimer’s disease alleviates amyloid-related pathologies in mice”


### Target Genes and Modified Organisms

“CRISPR base editing lowers cholesterol in monkeys”

autosomal dominant familial hypercholesterolemia, *PCSK9*

LNP delivery

---

**APP:** AMYLOID BETA A4 PRECURSOR PROTEIN

Modified adeno-associated virus (AAV) variants that can cross the blood–brain barrier (BBB)
**Target Genes and Modified Organisms**

"CRISPR-Based Adenine Editors Correct Nonsense Mutations in a Cystic Fibrosis Organoid Biobank" (2020)

"Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors"


Continuing progress on delivery to lung epithelial cells for therapy
Target Genes and Modified Organisms

“Transient CRISPR-Cas Treatment Can Prevent Reactivation of HIV-1 Replication in a Latently Infected T-Cell Line”

Fig. 1

Multiple short term edits (no introduction of DNA expression constructs or viruses, achieves loss of HIV function

Fig. 2

Using transposon delivery systems for CRISPR to target antibiotic resistance, transposons are what frequently spread resistance genes in bacteria populations.

## Target Genes and Modified Plants
### Plant Editing Firsts

<table>
<thead>
<tr>
<th>Plant</th>
<th>Species</th>
<th>Tech. notes</th>
<th>Gene(s)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>chickpea</td>
<td><em>Cicer arietinum</em></td>
<td>protoplasts</td>
<td>4CL7 RVE7</td>
<td>Drought Tolerance</td>
</tr>
<tr>
<td>rubber tree</td>
<td><em>Hevea brasiliensis</em></td>
<td>protoplasts</td>
<td><em>PDS</em></td>
<td>“Albinism”</td>
</tr>
<tr>
<td>hop</td>
<td><em>Humulus lupulus</em></td>
<td>Agro explants</td>
<td><em>PDS</em></td>
<td>“Albinism”</td>
</tr>
<tr>
<td><em>shēng di huáng</em></td>
<td><em>Rehmannia glutinosa</em></td>
<td>Agro explants</td>
<td><em>PDS</em></td>
<td>“Albinism”</td>
</tr>
<tr>
<td>oil palm</td>
<td><em>Elaeis guineensis</em></td>
<td>electro-transfection, biolistic particle</td>
<td><em>PDS BRI1</em></td>
<td>“Albinism” stunting</td>
</tr>
<tr>
<td>ergot fungus</td>
<td><em>Claviceps purpurea</em></td>
<td>Fungal pathogen</td>
<td><em>pyr4 and TrpE</em></td>
<td>Decreased virulence</td>
</tr>
</tbody>
</table>
## Target Genes and Modified Plants

### Notable Plant Edits

<table>
<thead>
<tr>
<th>Plant</th>
<th>Gene</th>
<th>Effect</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cherry tomato</td>
<td>eIF4E2 in a</td>
<td>resistance to pepper veinal mottle virus</td>
<td>increased yield</td>
</tr>
<tr>
<td>soy</td>
<td>NBS-LRR</td>
<td>Novel resistances</td>
<td>increased yield</td>
</tr>
<tr>
<td>tomato</td>
<td>MAX1</td>
<td>Resistance to parasitic root weed</td>
<td>increased yield</td>
</tr>
<tr>
<td>banana</td>
<td>DMR6</td>
<td>Resistance to banana Xanthomonas wilt</td>
<td>increased yield</td>
</tr>
<tr>
<td>potato</td>
<td>DND1, CHL1, DMR6</td>
<td>Resistance to late blight</td>
<td>increased yield</td>
</tr>
<tr>
<td>rapeseed</td>
<td>SHP1 and SHP2</td>
<td>Decreased shattering of seed pods during mechanical harvest</td>
<td>increased yield</td>
</tr>
<tr>
<td>grapefruit</td>
<td>LOB1</td>
<td>Resistance to canker</td>
<td>enhanced production</td>
</tr>
<tr>
<td>banana</td>
<td>ACO1</td>
<td>reduction of endogenous ethylene production</td>
<td>enhanced shelf life</td>
</tr>
<tr>
<td>grapevine</td>
<td>PAT1 (more)</td>
<td>Enhanced cold tolerance</td>
<td>env. stability</td>
</tr>
<tr>
<td>Rice</td>
<td>RLCK241 (more)</td>
<td>Enhanced salt tolerance</td>
<td>env. stability</td>
</tr>
</tbody>
</table>
Origin Story
Transposon Life Cycle

Transposase cuts element out and pastes into new location (no expansion in copy number)

Second gene in bacterial elements is dispensable and unknown function

https://en.wikipedia.org/wiki/DNA_transposon
RNA-guided transposons (mobile genetic elements) are the progenitors of Cas12 and Cas9

RNA-guided transposons (mobile genetic elements) are the progenitors of Cas12 and Cas9

Evolutionary Time


"Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease"

By reclaving its “sister-site” and promoting recombination, it has a “backup” in case the excised copy fails.

Also, explains how the copy number of the “non-replicative” transposon actually increases

2 copies to 3 copies

The Cas9 DSB can lead to a cascade of consequences for genome integrity which PERSIST in multiple generations of cells. These are hallmarks of cancer cells.

“Cas9 did what?!?”

“CRISPR-Cas9 globin editing can induce megabase-scale copy-neutral losses of heterozygosity in hematopoietic cells”

“Whole chromosome loss and genomic instability in mouse embryos after CRISPR-Cas9 genome editing”


Low immunogenicity means more injections of same gene editing but ALSO to treat multiple diseases.

“Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice”

Technology Advances

“Programmable deletion, replacement, integration and inversion of large DNA sequences with twin prime editing”

Allows for correction of a uniquely problematic and difficult class of genetic mutations

“Advances in Agrobacterium transformation and vector design result in high-frequency targeted gene insertion in maize”


Since 2016, 100-fold increase in quality transgenic plant development
Massive parallel edits in a population to screen for changes. Find improved bacteria and yeast for X. 

A push-button, benchtop biofoundry. The Onyx Digital Genome Engineering Instrument automates all aspects of large-scale, massively parallel genome engineering experiments — including the cell transformation, CRISPR-based genome engineering, cell growth, and cell recovery — all at your benchtop, all push-button easy.

https://www.inscripta.com/products/onyx-instrument

New Tools

“CRISPR-Associated Transposase System Can Insert Multiple Copies of Donor DNA into the Same Target Locus”

Single Delivery but with multiple payloads delivered into one site.
(only in bacteria so far)

"Find and cut-and-transfer (FiCAT) mammalian genome engineering"


"Drag-and-drop genome insertion without DNA cleavage with CRISPR-directed integrases"

Directed payload insertions (>30kb) and no double-strand breaks


**New Tools**

Smaller editing Cas proteins enable delivery on AAV

Also, new IP landscape

By comparison, Cas9 is 1500 aa
“A genome-wide CRISPR screen identifies host factors that regulate SARS-CoV-2 entry”

Genome screening technology – what promotes the survival of cells in face of infection?

Found genes that promote the endosomal pathway aided by ACE expression. Virus can be retransmitted.
New Tools

“Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing”

“dead” Cas9 with transcription activators and silencers demonstrated with multiple gene targets and inheritable in inducible pluripotent stem cells.

Applications include tissue engineering

Applications also include pharmacology

2021 CRISPR Year in Review

2021 CRISPR Year in Review

A very exciting year demonstrating the wide range of genome editing techniques and applications.

Questions?