HOMELAND SECURITY ADVISORY COUNCIL

FINAL REPORT OF THE EMERGING TECHNOLOGIES SUBCOMMITTEE

BIOTECHNOLOGY

AUGUST 18, 2020

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This publication is presented on behalf of the Homeland Security Advisory Council, Emerging Technologies Subcommittee, under Co-Chair Thad Allen, Co-Chair Cathy Lanier and Co-Chair Robert Rose as the ***final report*** and recommendations to the Acting Secretary of the Department of Homeland Security, Chad Wolf.

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EMERGING TECHNOLOGIES SUBCOMMITTEE

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EXECUTIVE SUMMARY - EMERGING TECHNOLOGIES SUBCOMMITTEE

The accelerated pace of the technological change in today's global research and development ecosystem is creating both risks and opportunities in the Department of Homeland Security's (DHS) mission domain. The dual challenge of addressing emerging technological threats to the Homeland while simultaneously acquiring and deploying capability to meet new threats is of paramount importance now and in the foreseeable future. Emerging technologies could pose threats for which no effective countermeasure readily exists, or they may comprise powerful new enabling capabilities that can be used by operational end-users. The problem is further exacerbated by evolving legal frameworks such as the recently passed FAA Reauthorization that provide new authorities but increase the complexity of implementation across the federal government and with DHS. In turn that complexity increases yet again when effective implementation of policy and deployment capability must be coordinated with state, local, tribal and territorial (SLTT) authorities.

To assist DHS in forecasting both threats and opportunities, work with partners, and improve the ability of DHS components to execute mission critical objectives, the Secretary chartered the Emerging Technologies Subcommittee of the Homeland Security Advisory Council (HSAC) in the Fall of 2018. The subcommittee was charged with exploring six emerging technologies and to develop recommendations to address and mitigate threats but also to take advantage of new capabilities to execute DHS missions. Those technologies include:

- Unmanned autonomous systems (UAS),
- Artificial intelligence and machine learning (AI/ML),
- 3/4D Printing
- Biotechnology – gene editing, splicing.
- Quantum information science and quantum computing.
- Advance Robotics

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RECOMMENDATIONS OF THE FINAL REPORT

Gene editing technology represents a major scientific advance that has the potential to greatly help or greatly harm the United States. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gives scientists the ability to manipulate DNA far beyond previous technology and has opened the door to rapid development in the field of molecular biology. However, the speed of innovation has outstripped American regulatory policy and legislation; given the paradigm altering potential of CRISPR and related technology, this disconnect must be closed. The following three recommendations address how the Department of Homeland Security should respond to ensure CRISPR is not used to harm the United States.

Recommendation #1

Actively get ahead of advances in CRISPR and gene therapy delivery systems. DHS should have a task force focused on developments in the field of CRISPR and its applications. CRISPR is an unprecedented technological advancement in molecular biology. It poses many benefits but also many threats. In the coming years, threats to the Homeland will develop from CRISPR genome manipulations. The committee tasked with monitoring the technology as it expands would predict witting and unwitting threats of CRISPR that might harm the health, food resources, and/or national interests of the United States. The committee could serve as a resource to propose government or international policies for engaging the technology responsibly. Such a committee would network with the leading CRISPR researchers and entities in government, academia, and industry to keep a close track on avenues of leading research, giving the committee the ability to anticipate threats that could be on the horizon.

Recommendation #2

At some point, it will become essential to determine whether CRISPR has been used, regardless of whether it was an accidental or intentional deployment of the technology. As the basic science of CRISPR is rapidly becoming a tool for genome modification, DHS should be concerned with also developing means to detect its use.

Recommendation #3

DHS should be monitoring and/or developing means to prevent the action of CRISPR technology or their delivery systems to prevent unwanted CRISPR modifications. For example, in the case of an accidental or intentional release of a gene drive that might harm U.S. citizens, U.S. food supply, vegetation, or wildlife, it may become necessary to understand mechanisms to inhibit the action of CRISPR technology.

EMERGING TECHNOLOGIES: BIOTECHNOLOGY

1. Assessment of the current state and perceived future advancements over the next 3-10 years that could pose a threat to the homeland security of the United States.

1.1 Current State of Gene Editing Technology

The emergence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) with CRISPR-associated endonuclease 9 (Cas9) is a disruptor technology in the field of molecular biology that allows rapid and precise modification of the genome at a fidelity that did not previously exist in molecular biology. The application of this technology represents a new capability in synthetic biology that renders most other gene editing capabilities instantly obsolete. Cumbersome gene editing experiments that once took weeks, months, or years to complete can be performed with less technical skill and effort at a fraction of the previous time and cost. While a technical hurdle still exists for performing CRISPR/Cas9-mediated genome edits, this technology lowers the bar to entry significantly. Fundamentally, the technology opens research avenues for manipulating DNA and represents a paradigm shift in the manipulation of biological systems at the molecular level. The potential implications of this new technology have led to a surge in molecular biology research compared to previous years. The sudden leap of capability has pushed beyond the current level of understanding of the CRISPR technical system, the appreciation of its lasting implications for genome modification applications, and the policies that govern those applications.

Technological advances in genome manipulation such as CRISPR allow the rapid and targeted modification of genomes *in vitro* and *in vivo*,[1] greatly increasing the speed and fidelity at which engineered genomic modifications can be made. To name a few examples, research focused on the manipulation of the genome sequence allows the development of novel gene therapies and drug target discovery, agricultural crop and livestock advancements, increases in accuracy and speed of basic scientific research to understand biological systems, and added options for control of emerging pathogen threats.

Although CRISPR technology is currently still in development, in terms of both application and basic scientific understanding, it represents a leap forward for genome engineering. The greatest advantages to the technology lie in the simplicity with which a genomic target sequence can be cut, requiring minimal molecular components to induce the DNA cut. For targeted genome modifications, donor DNA is also required to introduce the specific mutation or gene. As the scientific field actively works to understand the parameters surrounding this new molecular tool, limitations of the CRISPR technology are emerging. Unanticipated off-target cutting activity, in which CRISPR cuts unintended locations within the genome, have sparked research into the discovery of new Cas endonuclease enzymes and engineered modifications of known Cas enzymes to increase fidelity of the cutting activity, which would thereby reduce the risks of the technology to modify unintended genome regions. As with other potential gene therapies, CRISPR technology is also limited by deficiencies in

[1] *In vivo* studies are experiments, tests, or procedures done on or in a living organism such as a laboratory rat. *In vitro* studies are experiments or tests done in a test tube or petri dish.

effective cell delivery techniques that would move CRISPR components into all cells or targeted cell systems of a multicellular organism.

Acknowledging the imperfections of CRISPR, the technology represents a fundamental shift in genome engineering, bringing a new molecular tool to the laboratory bench that has instantly made other tools in the field less desirable or obsolete. The technology will continue to rapidly expand in the near future as the system is more completely defined and understood and new molecular applications and capabilities are developed.

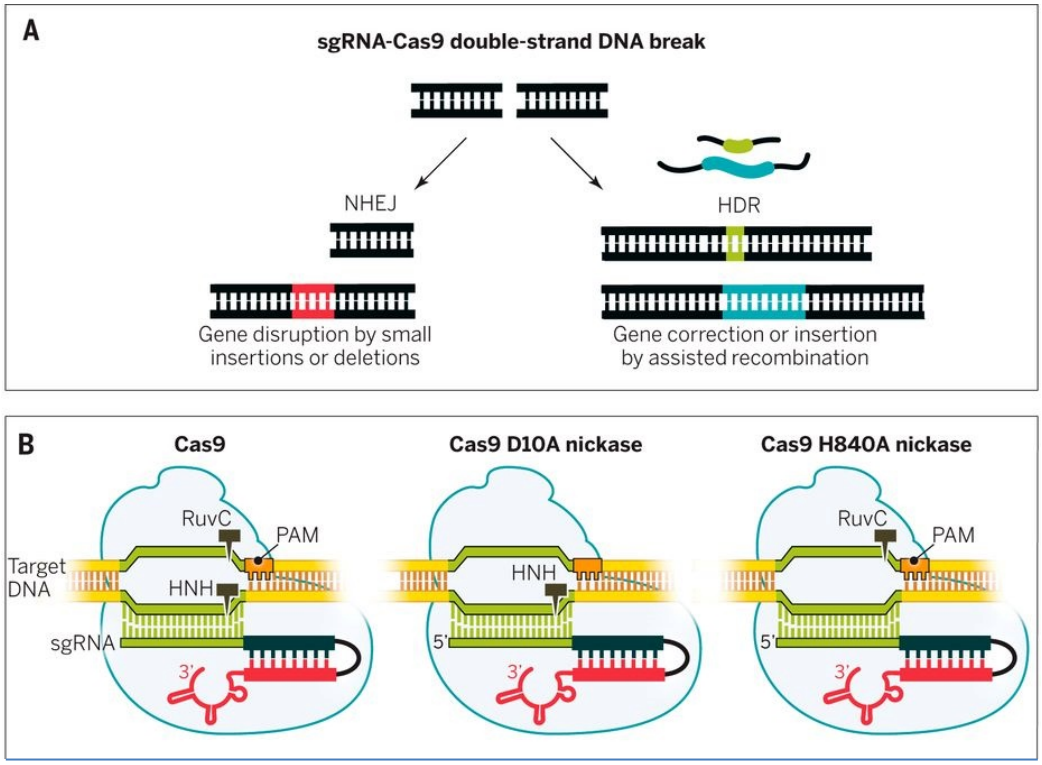
1.2 CRISPR Gene Editing Technology Overview

CRISPER/Cas9 represents a major leap forward in gene editing technology. These advances, however, would be impossible without the preceding discovery of Polymerase Chain Reaction (PCR). Developed in the 1980s, PCR allows scientists to make unlimited copies of DNA fragments from a single DNA molecule.[2] This reaction is critical to molecular biology research like gene editing because DNA is extremely delicate and difficult to isolate in consistent sections. With PCR, scientists can experiment and iterate with both speed and ease on identical sections of DNA code, allowing them to clearly isolate causal mechanisms. PCR paved the way for CRISPER/Cas9 by creating a relatively easy way to isolate specific gene and DNA sequences for a variety of entities and to test various treatment effects quickly and cheaply.

The rapidly emerging gene editing technology of CRISPR/Cas9 has the ability to produce precise sequence-targeted cleavages of DNA *in vitro* and *in vivo*. The precision of the endonuclease sequence cleavage mechanism is attributed to the genome sequence complementarity of the guide RNA directing the ribonucleoprotein complex for cleavage at the specific genomic location. In its simplicity, the CRISPR ribonucleoprotein consists of a guide RNA (gRNA) and a non-specific CRISPR-associated endonuclease (Cas) (Figure 10). The guide RNA in the CRISPR Technology contains a region of sequence that associates with the Cas endonuclease and a region of sequence that complements the sequence of the genomic target region. The Cas enzyme recognizes a specific protospacer adjacent motif (PAM) sequence in the genomic sequence. When the Cas associates with the PAM, and if the gRNA complements that genomic region, Cas9 is stimulated to make a double-stranded break about 3-4 base pairs upstream of the PAM sequence. For example, the PAM sequence recognized by Cas9 is NGG, with N being any of the four possible nucleotides followed by two guanine residues. The requirement for the PAM limits the precise locations that can be targeted. Cas9 currently has the most evaluation in the scientific literature; however, several Cas enzymes have been and continue to be discovered and developed for incorporation into the technology as it evolves.

[2] Mullis, K. (1990). The Unusual Origin of the Polymerase Chain Reaction. Scientific American, 262(4), 56-65. Retrieved July 7, 2020, from www.jstor.org/stable/24996713

Figure 10: CRISPR as a Genome Editing Technology



Upon insertion of a double-stranded break into the targeted region of the genome, the non-homologous end-joining (NHEJ) or homology-directed recombination (HDR) technique is used to insert the mutation into the genome (A). Cas9 complexes with the scaffolded sgRNA to form the ribonucleoprotein complex (RNP). The Cas9 of the RNP recognizes its specific protospacer adjacent motif (PAM) sequence within the genome. If the PAM is adjacent to a sequence that complements the gRNA sequence of the sgRNA, the complementary bases pair in a zipper-like manner that stimulates the RuvC and HNH catalytic sites to cleave both strands of the genomic DNA, 3-4 bases upstream of the PAM (B). The Cas9 enzyme can be engineered to have nickase activity, cleaving only one strand of the target genomic region. Cas9 D10A contains a mutation in the RuvC active site, while Cas9 H840A contains a mutation in the HNH active site (B). The image is modified from Doudna and Charpentier.[3]

The targeted genome breaks induced by CRISPR activate cellular repair mechanisms. During the process of repair of these nicks and double-stranded breaks within the genome, exogenous DNA molecules can be inserted randomly or in a directed manner. Random insertions using the non-homologous end-joining (NHEJ) technique are less predictable and result in uncontrolled insertion and deletion (indel) events. NHEJ is most useful when trying to knock-out gene function. Directed insertions using the homology-directed recombination

[3] Doudna JA, Charpentier E. 2014. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096.

(HDR) technique rely on the provided donor DNA to contain homologous arms with sequence homology to the genome, flanking each side of the desired sequence modification or insert. In this manner, the repair mechanism recognizes the donor DNA as more closely resembling the natural repair of the diploid genome and results in precise DNA insertions. The generation of highly efficient and precise double-stranded breaks is an essential prerequisite to attempt gene knock-out or knock-in assays using CRISPR.[4] According to Baud, *et al*., the simplicity and high efficiency of the CRISPR/Cas9 system makes it a very attractive alternative to traditional knockout procedures.[5]

1.3 Applications of Note

The introduction of CRISPR technology in 2012 was a game-changer for genomic manipulations, making them less challenging and more precise.[6] Since then, the technology has been utilized to induce DNA edits *in vitro* and *in vivo* across numerous biological organisms. The simplicity and efficiency of the application of CRISPR technology in seemingly any animal model are major advantages and may hold the future of animal model-based investigations of complex traits.[7] The speed with which a disease model can be constructed for a given organism may allow construction of very specific disease states to discover new, customized treatments. In addition, the methodology allows modification of multiple genomic loci in a single experiment, facilitating complex observations and affecting gene discovery through multiple mutation interactions. While the list of applications expands well beyond Figure 11, CRISPR applications in several common model systems are highlighted.

Figure 11: A Selection of Model Organisms with CRISPR Applications

Organism	Common Name	References
Homo sapiens	Human	(14, 18)
Drosophila melanogaster	Fruit Fly	(26, 27)
Ovis aries	Sheep	(28)
Glycine max	Soybean	(29, 30)
Triticum aestivum	Wheat	(5)
Arabidopsis thaliana	Arabidopsis	(31)
Danio rerio	Zebra Fish	(32, 33)

[4] Albadri S, Del Bene F, Revenu C. 2017. Genome editing using CRISPR/Cas9-based knock-in approaches in zebrafish. Methods doi:10.1016/j.ymeth.2017.03.005.

[5] Baud A, Flint J. 2017. Identifying genes for neurobehavioural traits in rodents: progress and pitfalls. Dis Model Mech 10:373-383.

[6] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337:816-821.

[7] Baud A, Flint J. 2017.

Caenorhadbitis elegans	Nematode	(34)
Ambystoma mexicanum	Salamander	(35)
Xenopus tropicalis	Frog	(36, 37)
Mus musculus	Mouse	(38-40)
Rattus rattus	Rat	(41, 42)
Sus scrofa	Pig	(43, 44)
Rhesus macaque	Monkey	(45)

1.4 Delivery Systems for CRISPR

CRISPR technology components must be delivered to the nucleus of the cell to induce genomic modifications. As with predecessor gene therapies, delivery systems that can move the CRISPR components efficiently and completely to targeted cells are lacking, which represents a technical hurdle. Once a path over this hurdle is discovered, many more avenues and applications for CRISPR will open. Current delivery strategies include physical delivery by microinjection or electroporation, viral delivery methods like adeno-associated virus (AAV), and non-viral delivery methods like liposomes, polyplexes, or gold particles.[8]

While the delivery systems employed today hold technical limitations for gene therapy applications and experimentation, they can still pose a threat. In one application published in 2014, an animal model for human lung cancer was develop in mice using CRISPR components packaged into adenovirus particles for delivery into the lung epithelial cells of the mice.[9] The CRISPR system was used to introduce breaks in two genes of the mouse chromosome 17 by co-expression of Cas9 endonuclease and two guide RNAs targeting the two sites. The Cas9 restriction in these two chromosome locations of the lung epithelial cells induced a flipped rearrangement of the internal sequence, mimicking similar cancerous Eml4-Alk inversion mutations observed in the human homologs of human chromosome 2.

Beyond the efficiencies observed in the induction of the cancerous Eml4-Alk inversion mutation within the mice, the use of adenovirus to deliver the CRISPR components to the lung epithelial cells is concerning. The application of the CRISPR components into the mouse lungs was facilitated by inhalation of the adenovirus, packaged with the CRISPR components. While the adenovirus used in this study was specific to the mouse model organism, human-specific adenoviruses exist and could be used for delivery of similar CRISPR components into human lung epithelial cells. With this study, the researchers not only demonstrated an efficient methodology for constructing a disease model within the mouse lung but also highlighted a delivery system that with minimal modification could be

[8] Lino CA, Harper JC, Carney JP, Timlin JA. 2018. Delivering CRISPR: a review of the challenges and approaches. Drug Delivery 25:1234-1257.

[9] Maddalo D, Manchado E, Concepcion CP, Bonetti C, Vidigal JA, Han YC, Ogrodowski P, Crippa A, Rekhtman N, de Stanchina E, Lowe SW, Ventura A. 2014. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature 516:423-427.

implemented with humans.

1.5 Modifying the Food Source – Livestock

Using CRISPR, a boost in the speed with which genomic modification can be introduced for genetic engineering of livestock genomes for use as food sources for the U.S. population. Traditionally, these types of transgenic animals undergo many years of scientific evaluation to ensure that they are safe for human consumption and have only recently gained traction for approval by the Food and Drug Administration (FDA). In 2015, the AquAdvantage Salmon, a transgenic animal with increased growth rate, became the first genetically engineered organism approved by the FDA for human consumption in the United States in a process that took six years after the FDA established guidelines for evaluation in 2009. The approval by the FDA of the AquAdvantage salmon as safe for human consumption opens a potential path for other genetically engineered food sources. The introduction of CRISPR technology provides a means to greatly increase the rate and the scope of transgenic animal production.

Breeding livestock to marketable and nutritional needs is a practice that is thousands of years old. Desirable traits are identified in offspring and encouraged through selective breeding practices. A massive and lucrative sector of business surrounds this practice and industry. The application of an effective genome editing technology like CRISPR would greatly shorten the time to achieve desired mutations or added traits within livestock. In one example, CRISPR has been used to manipulate the desired traits in the commercially valuable Shanbei cashmere goat. In a study by Wang, *et al*., the gene for fibroblast growth factor 5 (*FGF5*) was targeted for knock-out mutagenesis to increase the number of secondary hair follicles and the length of hair fibers in the goats. *FGF5* is the gene that controls fur length in the short- and long-haired Dachshund dog breed. The wild type state is to have the *FGF5* gene intact and functioning within the genome, producing a short-hair phenotype. When the gene is knocked out of the genome, the long-hair phenotype with increased follicle production is observed. Using CRISPR to create this knock out of the *FGF5* homolog within the goat genome, Wang, *et al*. produced cashmere goats with increased secondary hair follicles and longer hair fibers.[10] The resulting goats produced more marketable cashmere per individual goat.

A second application of CRISPR mutated the gene for myostatin (*MSTN*) in goats and sheep.[11] *MSTN* is associated with muscle development and inhibits muscle differentiation and growth. Selective breeding of cattle for a knock-out of functional myostatin produced the

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[10] Wang X, Cai B, Zhou J, Zhu H, Niu Y, Ma B, Yu H, Lei A, Yan H, Shen Q, Shi L, Zhao X, Hua J, Huang X, Qu L, Chen Y. 2016. Disruption of FGF5 in Cashmere Goats Using CRISPR/Cas9 Results in More Secondary Hair Follicles and Longer Fibers. PLoS One 11:e0164640; and, Wang X, Yu H, Lei A, Zhou J, Zeng W, Zhu H, Dong Z, Niu Y, Shi B, Cai B, Liu J, Huang S, Yan H, Zhao X, Zhou G, He X, Chen X, Yang Y, Jiang Y, Shi L, Tian X, Wang Y, Ma B, Huang X, Qu L, Chen Y. 2015. Generation of gene-modified goats targeting MSTN and FGF5 via zygote injection of CRISPR/Cas9 system. Sci Rep 5:13878.

[11] Wang X, et al., 2015; and, Crispo M, Mulet AP, Tesson L, Barrera N, Cuadro F, dos Santos-Neto PC, Nguyen TH, Creneguy A, Brusselle L, Anegon I, Menchaca A. 2015. Efficient Generation of Myostatin Knock-Out Sheep Using CRISPR/Cas9 Technology and Microinjection into Zygotes. PLoS One 10:e0136690.

double-muscled Belgian Blue and Piedmontese cattle lines.[12] Knocking-out this gene is known to cause hypertrophy of muscle mass in several mammal models, including mice, dogs, cattle, and humans.[13]

1.6 Modifying the Food Source – Agricultural Crops

Commodity crops have increased demands on their yields as the global human population increases. According to Doudna and Charpentier, the application of CRISPR technology to these crop plants promises to change the pace and course of agricultural research.[14] For example, the efficiency of CRISPR increases the yield of impactful genome manipulation (nearly 50 percent transformant yields) in rice, and these modifications are passed to progeny in a stable manner with few off-target editing events.[15] Doudna, *et al*. speculate that these findings point to CRISPR providing a method to genetically program protection from disease and resistance to pests in a manner that is superior to predecessor technologies. Development of technologies like CRISPR that facilitate rapid and precise genome editing in agricultural crops provide an opportunity for future food security.[16]

The technological advance of CRISPR Technology also side-steps a previously limiting factor for development of transgenic animals as food sources, as was observed in the modification of the white button mushroom. In April 2016, the United States Department of Agriculture (USDA) declined to regulate the cultivation and sale of the CRISPR-edited white button mushroom in the United States.[17] This decision made the mushroom the first CRISPR-modified organism to receive approval by the U.S. Government, but it also highlighted the reduction of a previous technical limitation for producing these type deletion edits. The CRISPR genome modification of the mushroom knocked-out six polyphenol oxidase (PPO) genes, which cause the caps of the mushrooms to brown, making them more desirable for sale for a longer period. The CRISPR-edited mushroom evaded the USDA regulatory process because it was not modified using foreign DNA from viruses or bacteria. When the U.S. Government developed the framework for regulating genetically modified organisms (GMOs) in the 1980s and 1990s, these organisms were necessary to implement these type genome

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[12] Kambadur R, Sharma M, Smith TP, Bass JJ. 1997. Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. Genome Res 7:910-916.

[13] robet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Menissier F, Massabanda J, Fries R, Hanset R, Georges M. 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat Genet 17:71-74; Kim JS, Petrella JK, Cross JM, Bamman MM. 2007. Load-mediated downregulation of myostatin mRNA is not sufficient to promote myofiber hypertrophy in humans: a cluster analysis. J Appl Physiol (1985) 103:1488-1495; McPherron AC, Lee SJ. 2002. Suppression of body fat accumulation in myostatin-deficient mice. J Clin Invest 109:595-601; and, Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellersh CS, Parker HG, Ostrander EA. 2007. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. PLoS Genet 3:e79.

[14] Doudna JA, Charpentier E. 2014.

[15] Zhang H, Zhang J, Wei P, Zhang B, Gou F, Feng Z, Mao Y, Yang L, Zhang H, Xu N, Zhu JK. 2014. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. Plant Biotechnol J 12:797-807.

[16] Georges F, Ray H. 2017. Genome editing of crops: A renewed opportunity for food security. GM Crops Food 8:1-12.

[17] Waltz E. 2016. Gene-edited CRISPR mushroom escapes US regulation. Nature 532:293.

modifications.[18] The advent of rapid and precise genome editing by CRISPR is forcing the U.S. Government to rethink its regulations on GMOs with regard to these advances in genome editing technologies, as the surge in their application is bringing a new generation of plant varietals to the market.[19] In March 2018, the USDA elaborated on its position in a published statement on plant breeding innovation.[20] The statement describes the application of gene editing technologies like CRISPR as plant breeding innovations that can introduce new plant traits more rapidly than traditional breeding techniques, potentially saving years or even decades from the introduction of new, robust plant varieties to farmers. In the statement, the USDA declines to provide oversight for the use of genetically altered plants, if the alterations could have been developed through traditional breeding methods likes cross-breeding and desirable trait selection. Transgenic plants that contain inserted genes from other species will continue to be regulated by the USDA.

1.7 Gene Drives

Gene drive is the introduction of a genetic trait or allele into a system with the added pressure that the introduced allele is favored over the wild-type allele. In natural breeding, a mutant allele along with a wild-type allele would be passed to progeny. In gene drive, the CRISPR components of Cas9 and the single guide RNA sequence, targeting the wild-type allele, are packaged into the mutant allele. Once the copy of the mutant allele is inherited, it actively cuts the wild-type sequence that is present, allowing the wild-type allele to be replaced by the mutated allele through recombinant repair mechanisms. In this manner, gene drive works best in organisms that reproduce sexually and have short generations.

Gene drives have been proposed as a method to control insect vectors that carry diseases like malaria, dengue, and Lyme. Dissemination of gene drives into the insect population might make the insects sterile and unable to replicate or disrupt the ability of the insect to transmit the disease. Applications of gene drive in herbicide resistant weeds that harm agricultural crops could be used to reverse their resistance mechanisms, making them once again susceptible to the herbicide.[21] Limitations of gene drives include their vulnerability to inactivation due to natural selection, especially if the gene drive produces a deleterious effect on the organism. A gene drive of this type would require continual monitoring and modification as resistance to the drive is developed within the population.[22] Further, gene drive has been described as a potential bioweapon that could be directed toward a population or its food supply. For example, a gene drive introduced into a commodity crop like corn or wheat could limit production of the crops. Gene drive could be used to target key insect

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[18] Ibid.

[19] Ledford H. 2016. Gene-editing surges as US rethinks regulations. Nature 532:158-159.

[20] USDA. 2018. Secretary Perdue Issues USDA Statement on Plant Breeding Innovation, Release No. 00070.18 ed. United States Department of Agriculture.

[21] Simon S, Otto M, Engelhard M. 2018. Synthetic gene drive: between continuity and novelty: Crucial differences between gene drive and genetically modified organisms require an adapted risk assessment for their use. EMBO reports 19:e45760.

[22] Ouagrham-Gormley SB, Vogel KM. 2016. Gene drives: The good, the bad, and the hype, *on* Bulletin of the Atomic Scientists. https://thebulletin.org/2016/10/gene-drives-the-good-the-bad-and-the-hype/. Accessed December 7, 2018.

pollinator species in order to decrease the numbers available to conduct pollination, thereby indirectly affecting the production of a wide number of crops that rely on insect pollination. In February 2016, James Clapper, the then-U.S. Director of National Intelligence, included gene editing in his annual Worldwide Threat Assessment report to the U.S. Congress as a global threat.[23]

1.8 Human Applications

Although CRISPR applications in the human genome have raised the greatest debate in recent years, many examples exist for the application of CRISPR in mammalian systems, leading to its application in humans. These types of applications within the human genome can be categorized as either somatic or germline edits. Genome edits within somatic cells typically affect localized regions or tissue types. The CRISPR components are delivered to the cells using a delivery system such as cationic lipid vesicles, gold particles, or adeno-associated virus.[24] Currently, clinical trials are underway to evaluate the utility of these types of edits for gene therapies in humans. Germline edits are performed very early in embryonic development or even during or before fertilization. The goal of this type edit is to modify the genome location in every cell of the organism. In this application, the CRISPR components are typically introduced using a microinjection technique directly into the nucleus of the zygote.[25] In humans, publications have described the application of germline edits in zygotes that are not allowed to develop into humans. Recently, a scientist from China claimed to have performed germline edits to knock-out the CCR5 gene of twin human girls; however, the controversial germline modifications within the genomes of the twins have not been independently verified. Regarding prospects as therapies, somatic edits and germline edits have their respective situational uses.

Cancer Biology

During the summer of 2017, the FDA completed a multi-year evaluation of the chimeric antigen receptor (CAR) T cell cancer therapy for application to blood cancers like

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[23] Clapper JR. 2016. Worldwide Threat Assessment of the U.S. Intelligence Community, *on* Office of the Director of National Intelligence. https://www.dni.gov/files/documents/SASC_Unclassified_2016_ATA_SFR_FINAL.pdf. Accessed December 7, 2018.

[24] Zuris JA, Thompson DB, Shu Y, Guilinger JP, Bessen JL, Hu JH, Maeder ML, Joung JK, Chen Z-Y, Liu DR. 2015. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. Nature biotechnology 33:73-80; Lee B, Lee K, Panda S, Gonzales-Rojas R, Chong A, Bugay V, Park HM, Brenner R, Murthy N, Lee HY. 2018. Nanoparticle delivery of CRISPR into the brain rescues a mouse model of fragile X syndrome from exaggerated repetitive behaviours. Nature Biomedical Engineering 2:497-507; and, Ehrke-Schulz E, Schiwon M, Leitner T, Dávid S, Bergmann T, Liu J, Ehrhardt A. 2017. CRISPR/Cas9 delivery with one single adenoviral vector devoid of all viral genes. Scientific Reports 7:17113.

[25] Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, Lv J, Xie X, Chen Y, Li Y, Sun Y, Bai Y, Songyang Z, Ma W, Zhou C, Huang J. 2015. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell 6:363-372; and, Ma H, Marti-Gutierrez N, Park S-W, Wu J, Lee Y, Suzuki K, Koski A, Ji D, Hayama T, Ahmed R, Darby H, Van Dyken C, Li Y, Kang E, Park AR, Kim D, Kim S-T, Gong J, Gu Y, Xu X, Battaglia D, Krieg SA, Lee DM, Wu DH, Wolf DP, Heitner SB, Belmonte JCI, Amato P, Kim J-S, Kaul S, Mitalipov S. 2017. Correction of a pathogenic gene mutation in human embryos. Nature advance online publication.

acute lymphoblastic leukemia, reviewed by Jackson, *et al*.[26] In this method of cancer treatment, T cells harvested from the patient are genetically modified to recognize markers on the specific cancer cells that have developed within the patient. This highly customized method relies on genomic modifications of the T cells from the patient. Researchers from Memorial Sloan Kettering Cancer Center have applied CRISPR in the mouse model to more rapidly and precisely introduce these genome modifications, providing an efficient means to introduce very specific mutations in a manner that increases the speed of the modification and does not exhaust the T cells.[27] The result is a CAR T cell lineage that contains the necessary targeting modifications but retains viability to be more effective against the cancer target.

Human Germline Editing

The application of CRISPR to humans immediately raises ethical concerns. CRISPR has no boundaries in the scope of its application and can be used to modify any genomic sequence. These genome edits are performed with unprecedented speed and accuracy and, if applied early in development as to modify all cells within the organism, are inheritable modifications that affect the germline of that organism. Human germline engineering represents permanent changes that are disseminated into the human population, influencing and shaping future generations. Members of the scientific community acknowledge that these modifications should be deliberate and that their potential impact should be fully considered. Further, most of the scientific community acknowledges that CRISPR is not understood well enough for use in human germline editing. With unpredictable phenomena like off-target cutting associated with the use of CRISPR, germline editing could introduce unintended mutations into the genome.

A moment of concern occurred following the 2015 publication of a Chinese study that applied CRISPR to the genomes of trinucleated zygotes.[28] This first publication regarding the application of CRISPR in human embryos was published in the journal *Protein and Cell* after being rejected by the journals *Nature* and *Science* due to its controversial application and the implications of human embryo editing.[29] In the study, Liang, *et al*., modified the endogenous β-globin gene (*HBB*) within zygotes. The result was a zygote with a mixture of both modified and unmodified cells. These results were deemed mixed and deficient by the greater scientific community. Although the efforts of Liang, *et al*., were considered a rush to apply CRISPR within humans, it was a first step in that direction. In late 2017, a study was published by Ma, *et al*., that built upon the findings of Liang, *et al*.[30] In the study, microinjection was used to act at an earlier stage of development than the Chinese study of 2015, decreasing the occurrence of mixed genotypes in the developing embryos.

In late November 2018, two days before the International Summit on Human Genome Editing in Hong Kong, China and in a disclosure that outraged many scientists based

[26] Jackson HJ, Rafiq S, Brentjens RJ. 2016. Driving CAR T-cells forward. Nat Rev Clin Oncol 13:370-383.
[27] Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, Odak A, Gonen M, Sadelain M. 2017. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 543:113-117.
[28] Liang P, et al., 2015.
[29] Cressey D, Cyranoski D. 2015. Gene editing poses challenges for journals. Nature 520:594.
[30] Ma H, et al., 2017.

on its disregard for ethical concerns, a Chinese scientist named He Jiankui disclosed that he had CRISPR-modified the genomes of twin human babies. In his work, he used CRISPR technology to knock-out the C-C chemokine receptor type 5 (CCR5) gene in early embryos. The CCR5 receptor gene has been linked to the susceptibility of human immunodeficiency virus (HIV) to enter the cell.[31] If validated, the twins are the first CRISPR-edited humans. Because He performed these experiments largely in secret, the announcement was followed by an international outcry denouncing the work as careless. The particular genome modification is seen as largely unnecessary with modern HIV treatments that control the virus, and there is concern that off-target modifications could have been introduced into the genomes unintentionally. While the summit was meant to bring together the international scientific community to debate and discuss human genome editing and the ethical considerations of it, the careless and self-driven actions of He were largely greeted with rebuke from the scientific community. His claims and the results of his studies have not been confirmed or vetted to determine if the edits were actually conducted and if they were safe; however, his secretive approach to conducting human genomic modifications cannot be denied. Further, it highlights the threat that scientists can use CRISPR to quietly modify the human germline with no oversight from the greater scientific community.

1.9 The Evolution of CRISPR Technology

To overcome the limitations of Cas9 endonuclease, scientists have begun to develop variants of Cas for gene therapies. Researchers have modified Cas9 into single base editors, eliminating the need to supply donor DNA to introduce a point mutation, and they have developed Cpf1 endonuclease, also known as Cas12a, that has increased sequence specificity over Cas9 and tolerates only a few mismatches to the genome complementary sequence within the single guide RNA sequence.[32] Cpf1 provides an option for decreasing the occurrence of off-target cutting events that could erroneously knock-out an unintended gene target.

The scientific community is only just beginning to develop the CRISPR technology. New enzymes and capabilities like single base editors and Cpf1 endonuclease will continue to emerge as discoveries and innovations in the usage of CRISPR are defined and developed. The drive to overcome the limitations of Cas9 endonuclease for the use of CRISPR technology in gene therapies will continue to fuel innovation in this area. For example, in the spring of 2018, Hu, *et al*., reported the development of xCas9 through phage-assisted continuous evolution, expanding the protospacer adjacent motif (PAM) sites that the enzyme recognizes. The resulting xCas9 enzyme can recognize a broad range of PAM sequences.[33] The modification of Cas9 in this way opens much more of the genome for editing, increasing the specificity of CRISPR by increasing the number of sites that are available to be cut.

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[31] Lopalco L. 2010. CCR5: From Natural Resistance to a New Anti-HIV Strategy. Viruses 2:574-600.

[32] Eid A, Alshareef S, Mahfouz MM. 2018. CRISPR base editors: genome editing without double-stranded breaks. The Biochemical journal 475:1955-1964; and, Strohkendl I, Saifuddin FA, Rybarski JR, Finkelstein IJ, Russell R. 2018. Kinetic Basis for DNA Target Specificity of CRISPR-Cas12a. Mol Cell 71:816-824.e813.

[33] Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, Zeina CM, Gao X, Rees HA, Lin Z, Liu DR. 2018. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 556:57.

1.10 Expected Advancements of Technology

New Cas Enzymes and Engineered Capabilities

CRISPR technology is in its infancy, and researchers have only begun to characterize the scope of what CRISPR can do or become. Over the next several years, as the basic science of CRISPR Technology is characterized, new Cas enzymes with varying capabilities will emerge. These capabilities will include more accurate cutting with fewer off-target cuts, resulting in increased fidelity and precision of genomic modifications. Acquiring this fidelity in genome cutting is being driven by the prospect of using CRISPR as a gene therapy to treat rare diseases and reverse cancerous mutations.

As was seen in the development of Cas nickase enzyme, the protein fusions of the single base editors, and the expansion of PAM recognition sites in xCas9 enzyme, scientists will not only leverage the naturally occurring Cas enzymes, but will engineer new modalities within the available suite of enzymes.[34] These advances in the molecular engineering of CRISPR will allow scientists to develop capabilities beyond those seen in natural systems.

Developing New Applications for CRISPR Technology

A few alarming applications have driven an ongoing conversation around CRISPR technology as it applies to safety and, more importantly, preservation of the human germline. CRISPR provides a means to drive evolution with efficiencies that have not been previously available. Combining this newly acquired power with the desires of individual or rogue scientists to "be the first" in their applications, the scientific community is struggling to restrain and self-govern itself. As was seen with the claimed CRISPR modification of two human babies in China in late November 2018, it is anticipated that more unsanctioned applications of CRISPR will occur in the future. That the scientist in that case conducted his work secretively without oversight of fellow scientists speaks to the profound power of CRISPR and the potential threat of those that will wield it for their own gain. The highly debated concept of designer humans is obtainable and not far from reality. The greater majority of the scientific community will move with caution and strive to apply CRISPR toward improving the human condition. The emergent threat, as was highlighted with the announcement of the CRISPR-edited human babies, is from those scientists who would conduct their experiments in secret with no oversight, while single-handedly modifying the heritable germline of the human species. Though it has not yet been seen, rogue countries could modify humans and other organisms to serve their own interests, which may run counter to U.S. interests.

CRISPR represents a groundbreaking technology for developing clinical treatments for the prevention of diseases and curing heritable and non-heritable genetic diseases. Limited by delivery systems for targeting specific cells or tissues, scientists will, in the near future, continue to develop cleaver means to apply CRISPR as potential methods for gene therapies. Breakthroughs in delivery systems will be quickly adopted by this scientific group to facilitate

[34] Ran FA, Hsu PD, Lin CY, Gootenberg JS, Konermann S, Trevino AE, Scott DA, Inoue A, Matoba S, Zhang Y, Zhang F. 2013. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154:1380-1389; Eid A, et al., 2018; and, Hu JH, 2018.

and expand their respective applications.

Synthetic gene drive systems using CRISPR have the potential to knock down the number of pests like malaria-carrying mosquitos and to contain other vector-borne diseases such as dengue, Lyme, and Zika.[35] Gene drives are also being applied to contain the emergence of drug resistance in *Candida albicans*, a human pathogen that is the leading cause of fungal infections.[36] Further, gene drives have been proposed as strategies to control unwanted and invasive weed species that deplete nutrients from commodity agricultural crops, making them more sensitive to herbicides.[37] In a report published in July 2018, strategies for gene drive technologies are now moving into experiments in rapidly reproducing mammalian systems.[38] Because gene drives have the ability to address vectors of human and animal disease and increase production in profitable agriculture crops while limiting the development of increasingly toxic herbicides, research and application into synthetic gene drive systems will continue in the near future. With the threat of the use of gene drives as bioweapons that could cripple the food resources of a nation or alter whole ecosystems, monitoring the advancements in gene drives will be necessary.

Development of Detection and Inhibition Capabilities

CRISPR technology has the ability to reach deep into the biological thread of society—to the core of the information that makes us the human species. It can enhance human health and food supplies while providing a means to sustain humanity and reduce human suffering. The tremendous advantages to be gained from CRISPR will continue its feverish drive toward innovation and discovery; however, the risks and threats associated with these rapid developments cannot be denied. Methods for inhibiting or controlling the activity of Cas enzymes will be critical. In January 2017, Maji, *et al*., reported a multidimensional chemical control of Cas9 that was modified with a fusion to a small molecule-dependent destabilized domain.[39] The protein fusion makes the Cas enzyme active only in the concentration-dependent presence of small molecules that can bind the destabilized domains. While this is a means to control Cas activity and may have applications in timing or spatially sensitive edits, this approach requires that the Cas is fused to the destabilized domain and would not have applications to unmodified Cas. Research groups at Harvard University and Sandia National Laboratories are actively searching for small molecules that would inhibit Cas9 activity.

With the expansion of applications for CRISPR and the recent birth of allegedly

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[35] Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. 2015. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito <em>Anopheles stephensi</em&gt. Proceedings of the National Academy of Sciences 112:E6736.

[36] Shapiro RS, Chavez A, Porter CBM, Hamblin M, Kaas CS, DiCarlo JE, Zeng G, Xu X, Revtovich AV, Kirienko NV, Wang Y, Church GM, Collins JJ. 2018. A CRISPR–Cas9-based gene drive platform for genetic interaction analysis in Candida albicans. Nature Microbiology 3:73-82.

[37] Simon S, et al., 2018.

[38] Grunwald HA, Gantz VM, Poplawski G, Xu X-rS, Bier E, Cooper KL. 2018. Super-Mendelian inheritance mediated by CRISPR/Cas9 in the female mouse germline. bioRxiv doi:10.1101/362558:362558.

[39] Maji B, Moore CL, Zetsche B, Volz SE, Zhang F, Shoulders MD, Choudhary A. 2017. Multidimensional chemical control of CRISPR-Cas9. Nature chemical biology 13:9-11.

RISPR-modified human babies, it will be critical for determining the cues that CRISPR has been employed to direct a mutation event. Making this determination will help investigators understand if a biological attack has been deployed or if a naturally occurring mutation has emerged. For example, in the case of CRISPR components delivered to the epithelial cells of mouse lungs to induce oncogenic mutations and resulting in cancerous tumors within the lungs, how would one determine that CRISPR was employed to cause an oncogenic mutation if these mutations were induced in human lungs using a similar inhalation-based viral delivery system? Looking further into the future and considering recent claims of the birth of the first CRISPR-modified humans, it may become necessary to determine if a human has been biologically enhanced using CRISPR.

Advancements in CRISPR Delivery Systems

The substrate of Cas nuclease activity is DNA, which is located within the nucleus of the cell. To reach its substrate, the enzyme or the genetic template for the enzyme along with the single guide RNA and donor DNA require a delivery system to reach the nucleus. While there are a few options for delivery systems, they are not precise or efficient. Delivery systems have historically been a limitation for gene therapies; however, with the advent of the simple yet precise CRISPR technology, there is increased demand for delivery systems. Rapidly making the precise edit is no longer the limiting factor; moving the CRISPR components into the correct vicinity is. The drive to use CRISPR in potential gene therapies will stimulate research in delivery system development. In turn, advancements in delivery systems will widen the scope of CRISPR applications.

One promising area of CRISPR component delivery centers on nanomaterials. Nanoparticles or nanocapsules have been found to be capable of carrying CRISPR components to cell nuclei, eliminating the need for less precise carriers like viruses. Nanocapsules are constructed from a thin polymer shell which protects the CRISPER enzyme and guide RNA from degradation in the bloodstream; once inside the target cell, the polymer degrades from exposure to glutathione (a molecule found at high levels within cells).[40] Using a non-viral carrier eliminates the possibility the patient has or will develop antibodies or will have a negative immune reaction, which would prevent delivery.[41] Further, nanomaterials can be 'programed' to deliver their CRISPR edits to specific types of cells, versus viral carriers which affect all cells. Finally, from a functional perspective, nanomaterials can be powdered, making their transport, storage, and administration far easier than viral carriers.[42] Nanoparticles have been found to be able to edit up to 80% of human cells with limited toxicity, making this a critical advancement in CRISPR utility.[43]

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[40] Collins, F. September 17, 2019. Nano-Sized Solution to Efficient and Versatile CRISPR Gene Editing. National Institute of Health. https://directorsblog.nih.gov/2019/09/17/nano-sized-solution-for-efficient-and-versatile-crispr-gene-editing. Accessed July 7, 2020.

[41] Trafton, A. November 13, 2017. CRISPR-carrying nanoparticles edit the genome. MIT News. http://news.mit.edu/2017/crispr-carrying-nanoparticles-edit-genome-1113. Accessed July 6, 2020.

[42] Collins, F. 2019.

[43] Chen, G., Abdeen, A. A., Wang, Y., Shahi, P. K., Robertson, S., Xie, R., Suzuki, M., Pattnaik, B. R., Saha, K., & Gong, S. (2019). A biodegradable nanocapsule delivers a Cas9 ribonucleoprotein complex for in vivo genome editing. Nature nanotechnology, 14(10), 974–980. https://doi.org/10.1038/s41565-019-0539-2

Impediments that Could Delay or Stall Deployment of Technology

CRISPR technology has arrived and is aggressively under investigation by scientists around the world. The technology has proven itself to be a useful tool for expediting gene modification steps, subsequently opening unprecedented options for genome modification. New aspects of CRISPR or new scientific steps taken using the technology are described almost monthly in journal publications. Advancements using the technology frequently appear in mainstream news reports, making it clear that CRISPR will be a part of molecular biology and affect the lives of individuals for the foreseeable future. The promise of the applications and advances in science that could be facilitated by the technology are opening sources of funding to fuel the research. In January 2018, the National Institutes of Health (NIH) announced the Somatic Cell Genome Editing Program, which will provide funding to researchers at approximately $190 million over the next six years.[44] Further, numerous companies have emerged around the technology including Editas Medicine, Inc., Intellia Therapeutics, and Caribou Biosciences, Inc., all grappling over intellectual property rights. Researchers across academia are leveraging CRISPR in their laboratories or engaging fee-for-service companies like Sigma-Aldrich or Genscript to design their CRISPR research strategies for use in their laboratories. The aggressive and rapid pace that CRISPR is being unpacked and the amount of resources directed at the research are not limiting factors for the development of the technology. Business Wire forecasts that CRISPR technology investments will grow rapidly from $550M (2017) to greater than $3B in 2023.

Limitations on the technology could come in the form of policy constrictions. When considering the application of CRISPR genome editing technologies, ethical considerations are an immediate concern. How should society approach a technology that can, with unprecedented speed and accuracy, modify the germline of species in a permanent manner that can be passed to future generations and disseminated into the population of that species? The power of CRISPR escalated rapidly to the first application in human embryos, leaping in front of regulations and highlighting the need for oversight. Following the April 2015 publication of the results from CRISPR editing in human embryo genomes reported from China, a moratorium on the application of gene editing technologies in the clinical setting on the human germline was called at the *International Summit on Human Gene Editing* at the National Academy of Sciences in December 2015.[45] This type of moratorium is an attempt by scientists to self-regulate; however, as was seen with the recent use of CIRSPR by Jiankui, a moratorium will not be followed by all scientists when the perception of scientific glory is attainable. In November 2018, the United Nations (U.N.) signed a treaty that agreed to limit the use of gene drives but rejected a moratorium on the development of the technology.[46] The U.N. acknowledged the restraint that needs to be implemented but also recognized the enormous value that gene drive represents. The vague nature of the treaty has been seen as more of a non-declaration of a stance on gene drives. Further, the USDA has clarified its regulatory

[44] Britt R. January 23, 2018. NIH to launch genome editing research program, *on* National Institutes of Health. https://www.nih.gov/news-events/news-releases/nih-launch-genome-editing-research-program. Accessed December 12, 2018.

[45] Liang P, et al., 2015.

[46] Callaway E. November 30, 2018. UN treaty agrees to limit gene drives but rejects a moratorium, on Nature. https://www.nature.com/articles/d41586-018-07600-w. Accessed December 12, 2018.

stance by allowing CRISPR genome edits of agricultural crops that could also be achieved by natural breeding processes. The topic of CRISPR technology and its application is under debate and review; however, no significant regulations restrict its use. Without the development of any formal restrictions, technological development is likely to outpace the policy constrictions.

Currently, the pace of discovery in CRISPR is held by the research itself. In many aspects, CRISPR is uncharted territory. Technical hurdles with the technology exist, which researchers must discover or engineer methods to overcome. One example is the frequency of off-target cutting observed with Cas9. The ability of researchers to conscientiously define new aspects and applications of CRISPR becomes a pace-limiting factor. As the rogue and secretive application of CRISPR to modify the genome of human babies continues to undergo review, scientists are monitoring how policy makers, funding agencies, and the world will react to the researchers involved with this secretive application of CRISPR that affects all humans. The reaction to and subsequent consequences of performing the experiments will define the level of impedance that is placed on other researchers that might do the same thing.

How Convergence of Other Emerging Technologies Could Increase Threat/Opportunity

As with other predecessor genome editing technologies, delivery systems that would move the CRISPR components into the nucleus of appropriate cells for directing genome edits are a limiting factor but also represent a close kinship to the technology. As new delivery systems emerge, they will provide the vehicle for opening applications of CRISPR technology. Similarly, advances in replicative gene drives to better utilize CRISPR technology for selfish allele advancement into the population will bolster the impact of gene drives. For example, a gene drive that evades adaptive mutation resistance within populations would extend the effectiveness of the gene drive for longer periods of time and deeper into the target population.

In emerging fields not typically associated with molecular biology, mechanisms for mechanical delivery systems can be imagined. For example, an unmanned aircraft system (UAS) could be used to disperse adenovirus particles packaged with mutation-causing CRISPR components across a crowd of people.

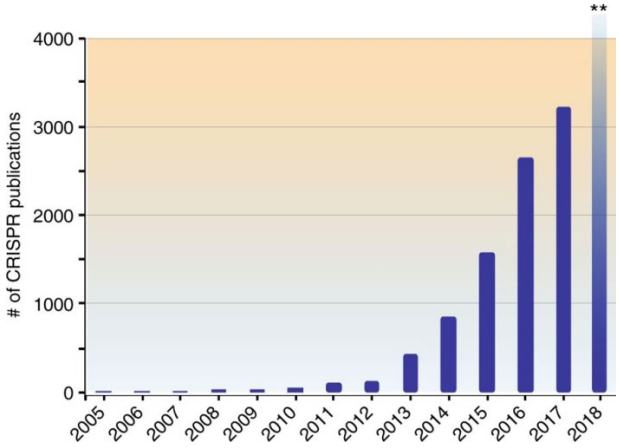
Dissemination of new achievements and milestones in CRISPR research occur at lightning speed. Advanced communications are the most sophisticated in the history of humanity. The development of occurring events are known rapidly around the globe. CRISPR is frequently described as a paradigm shift in molecular biology that is similar to that of polymerase chain reaction (PCR) in the early 1980s. That shift occurred without the presence of the Internet and modern communications. New scientific discoveries at that time were published in printed journal articles that could take weeks or months to review prior to publication. Developments were revealed at a slower, digestible rate. The CRISPR paradigm shift is unfolding in the modern communication age where research is released ahead of formal journal publication, which is for the most part all electronic now. In some cases, the announcement of scientific information is self-published by the scientist in alternative formats like *ReseachGate*, bypassing peer review processes. Internet forums centered on a given topic of research cater to a range of scientists from amateurs to experts; however, the information is available to all. Whole podcasts, like *CRISPR Cuts,* are centered on CRISPR technology. These podcasts are meant to be easily digestible and disseminate knowledge of CRISPR. The term and concept of a podcast did not even exist in the 1980s. Similarly, Twitter feeds like *CRISPR News* are solely devoted to rapidly reporting the newest developments in CRISPR. The vehicles for rapid dissemination of scientific information are drastically different from the

emergence of PCR in the 1980s. The presence of this multifaceted platform for monitoring what seems like real-time news in the field of CRISPR unifies the research community but makes predicting next steps and determining who has what information available to them more difficult.

Projected Timeline for Deployment of Technology

CRISPR technology is emerging now. Innovation in this field is advancing in multiple areas of molecular biology with numerous applications. Figure 12 illustrates the surge of research publications surrounding CRISPR over the past 12 years. The breakthrough capability that this technology allows will affect many aspects of life within the homeland including food products, healthcare, and national defense. Figure 13 illustrates the timeline of development for CRISPR with a rapid expansion after its application in eukaryotic cells in 2013.

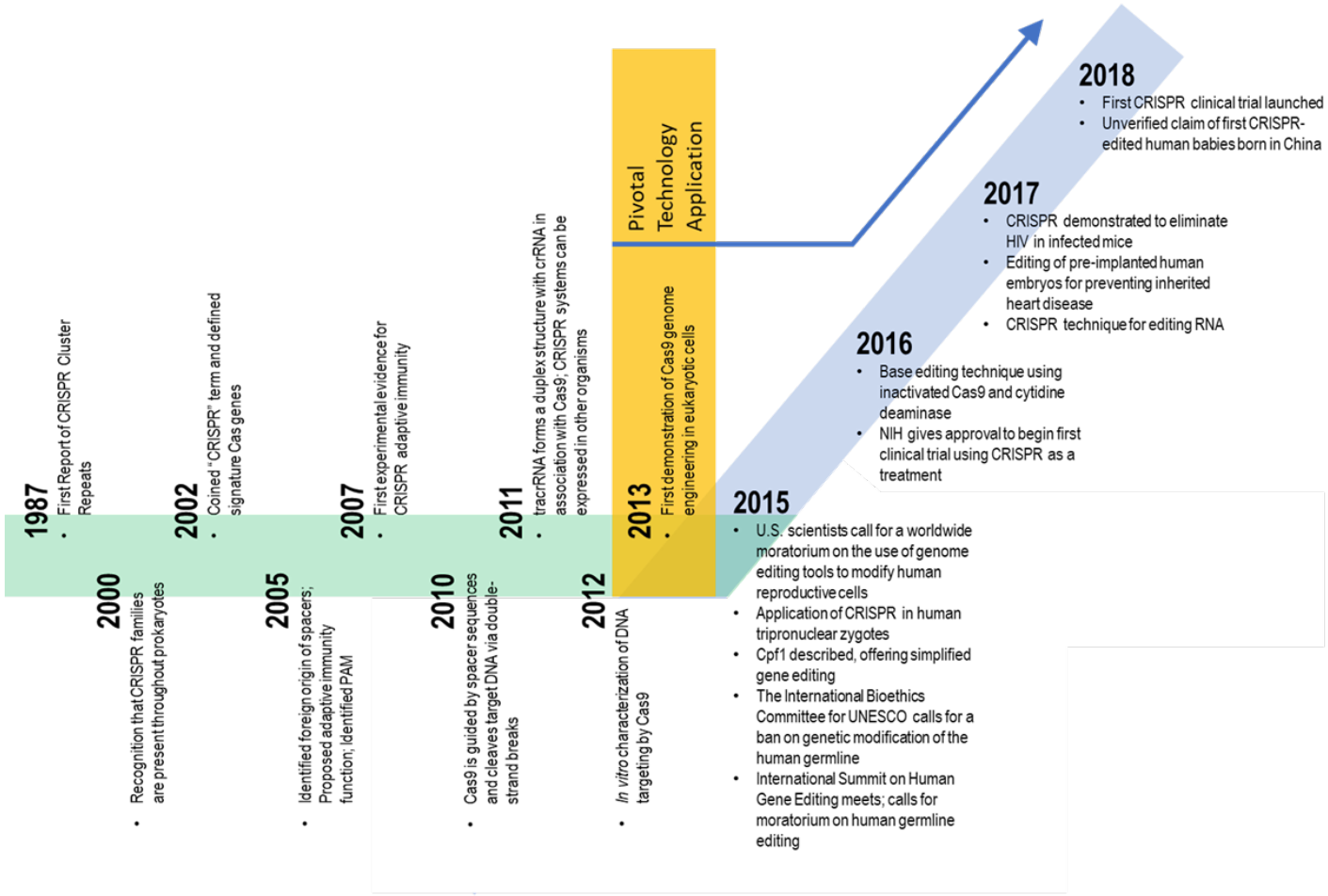
Figure 12: CRISPR Journal Publications



The number of publications within PubMed over the last 12 years that have the word "CRISPR" or "Cas9" in the abstract or title has exponentially grown since its application as a technology in 2012. **Projections for the number of publications in 2018 are estimated to be more than 5000. Image from *Nature Communications*.[47]

[47] Adli M. 2018. The CRISPR tool kit for genome editing and beyond. Nature Communications 9:1911.

Figure 13: CRISPR Timeline



As CRISPR Technology has developed, key points within its path have defined its use for editing the genome. Initially, basic scientific discoveries and characterizations of CRISPR components were distributed across the 2000s. After the demonstration of CRISPR Technology in eukaryotic cells in 2013, the applications of CRISPR have rapidly expanded. The illustration is modified from that of Norman, *et al*. and CRISPR-Cas9: Timeline of Key Events.

2. How such technologies could endanger the homeland, with a focus on those which have the highest likelihood of becoming a threat and those that pose the highest consequences to U.S. homeland security

2.1 New Capability for Homeland Security

Use Case #1

CRISPR technology could be used to control pathogens and pests in livestock and crops. For example, modification of the livestock genome to subvert the mechanisms of pathogenesis utilized by microbial pathogens could increase yields of livestock production. Gene drive utilizing CRISPR technology is currently under debate for use in targeting herbicide resistance factors that have developed over time in weed plants that deplete nutrients from agricultural crops. By knocking down herbicide resistance in the weeds, the development of increasingly toxic herbicides is avoided, and resources are not diverted away from crops.

Use Case #2

CRISPR technology is gaining traction for use as a gene therapy for genetic and pathogenic diseases. For example, a version of CRISPR might one day be used to correct cancer mutations in tumor cells or eliminate retroviruses like HIV from infected human genomes. Currently, the basic science aspects of CRISPR are not fully understood or predictable as seen in the case of the occurrence of off-target cutting.

Use Case #3

Coupling CRISPR to that of gene drive provides a potential capability to control insect vectors that spread disease. For example, using gene drive to knock down numbers of mosquitos in malaria-prone regions of the world can result in the control of malarial outbreaks. This type of vector control can be applied to insects that distribute Lyme, Zika, and other diseases to humans and livestock. Any interference with biological entities or specific populations within a wider ecosystem, however, could have dramatic second and third order effects and it is difficult to anticipate what these unintended consequences might be.

2.2 New Threat to Homeland Security

Use Case #1

CRISPR technology could be used to induce a mutation into a population or crowd of people. For example, CRISPR has been used to induce a cancerous mutation within the epithelial cells of the lungs of mice by packaging the CRISPR components for the mutation into mouse adenovirus particles. Upon inhalation, the adenovirus bound to receptors on the lung epithelial cells and delivered the CRISPR components to the nucleus of the cell.[48] A similar delivery method could be developed to affect humans using the human adenovirus to package and deliver the CRISPR components. Because the virus is inhalable, the release of the particle could affect many individuals in a given release area. Similarly, the particles could be released into air handling systems to affect whole buildings.

[48] Maddalo D, et al., 2014.

Use Case #2

Gene drive with CRISPR technology could be used to propagate a mutation into livestock or agricultural crops to weaken the food supply of the homeland. In a process similar to how gene drive could be used to make weed plants sensitive to herbicides or prevent insect pests from reproducing, a mutation could be introduced with gene drive that weakens or eradicates a species. Beyond the most extreme example of directly targeting a plant or animal, the viability of plants could be influenced indirectly by introducing a gene drive that eradicates critical pollinator species like honeybees.

Use Case #3

CRISPR technology could be used to rapidly mutagenize or manipulate the genomes of pathogens to quickly escalate pathogenicity. For example, seasonal influenza results from antigenic drift within the virus as it replicates and is passed from host to host. Periodically in history, the influenza virus has experienced an antigenic shift when it crosses species. An example would be in agrarian societies where humans work closely with livestock that can also contract influenza. The antigenic shift is a dramatic variation from what the immune system has seen in previous seasonal strains and can cause harmful pathogenicity and mortality as a result. CRISPR could be used to develop a shifted strain of influenza virus that a nation state could vaccinate their own population against prior to releasing the shifted strain. Similarly, genome editing could be used to change or enhance virulence factors of known pathogens or even bacteria or viruses that are not typically considered pathogens. For example, a non-pathogenic strain of anthrax, commonly found in nature, could be converted into a highly virulent form by altering its genome.[49] Others have raised the concern of whether CRISPR could be used to introduce antibiotic resistance into a bioweapons agent or to develop chimeric bioweapons that cause symptoms of one disease but attack the body with a different, undetectable disease.[50]

Use Case #4

With the declaration of the application of CRISPR technology in humans to modify the germline, it should be anticipated that other scientists and potentially rogue states would secretively perform similar experiments to modify genetic material in an effort to leap ahead of the understanding of the technology. While the directed evolution of the human species in this manner has the ability to help the human condition and eradicate certain types of diseases, the idea of developing designer humans with abnormal strength or advanced intelligence has been suggested by others and should be considered a threat.

[49] Gerstein DM. 2016. How genetic editing became a national security threat, *on* Bulletin of the Atomic Scientists. https://thebulletin.org/2016/10/gene-drives-the-good-the-bad-and-the-hype/. Accessed December 7, 2018.

[50] Vogel KM, Ouagrham-Gormley SB. 2018. Anticipating emerging biotechnology threats: A case study of CRISPR. Politics and the Life Sciences 37:203-219.

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APPENDIX A – PANEL MEMBER BIOGRAPHIES

Thad W. Allen (Co-Chair)

Thad Allen is a retired Admiral of the U.S. Coast Guard. He is currently the Executive Vice President at Booz Allen Hamilton and is a national thought leader and strategist in homeland security, maritime security, disaster response and recovery, and energy. He is known for his expertise in public-private sector collaborative efforts to improve national resiliency and create whole of community solutions to complex man-made and natural disasters. Allen completed his distinguished thirty-nine-year career in the U.S. Coast Guard as its 23rd Commandant in May 2010, when President Barack Obama selected him to serve as the National Incident Commander for the unified response to the Deepwater Horizon oil spill in the Gulf of Mexico. Prior to his assignment as Commandant, he served as Coast Guard Chief of Staff. During his tenure in that position, he was designated Principal Federal Official for the U.S. government's response and recovery operations in the aftermath of Hurricanes Katrina and Rita. For his service in those responses, Admiral Allen was the first recipient of the Homeland Security Distinguished Service Medal. Allen also currently serves as a director on the Coast Guard Foundation and Partnership for Public Service, a Fellow in the National Academy of Public Administration, and a Member on the Council on Foreign Relations.

Cathy Lanier (Co-Chair)

Cathy Lanier is currently the Senior Vice President and Chief Security Officer for the National Football League. She previously served as the Chief of Police with the Washington, DC Metropolitan Police Department (MPD) from 2007 to 2016. Ms. Lanier also served as the Commanding Officer of the Department's Major Narcotics Branch and Vehicular Homicide Units. In 2006, the MPD's Office of Homeland Security and Counter-Terrorism (OHSCT) was created, and Chief Lanier was tapped to be its first Commanding Officer. Ms. Lanier is a highly respected professional in the areas of homeland security and community policing. She took the lead role in developing and implementing coordinated counter-terrorism strategies for all units within the MPD and launched the department's Operation TIPP (Terrorist Incident Prevention Program).

Robert Rose (Co-Chair)

Robert Rose is a recognized expert providing the U.S. government and companies strategic counseling and governance on a full array of cyber-related issues at the nexus of technology, national security, law enforcement and privacy. Bob serves in various advisory positions in the areas of national security, cybersecurity, and homeland security. He currently serves as Senior Advisor to the Chairman and as an Advisory Board member for both 1Kosmos and Securonix. Bob is a member of the U.S. Department of Homeland Security's Homeland Security Advisory Council. Additional corporate and non-profit advisory board service include The Chertoff Group, MITRE's Homeland Security Experts Group, Cyber Florida, Opora, Plurilock, and the Council of Executives for Auburn University's Cyber and Homeland Security. Bob previously served as a senior advisor to the Chairman of Bridgewater Associates, and received appointments to the National Security Agency's Cyber Awareness and Response Panel, the Department of State's International Security Advisory Board, the National Counterterrorism Center's (NCTC) Advisory Board, and the Director of National Intelligence's Financial Sector Advisory Board. Bob has received numerous honors and awards, including: a presidential appointment to the J. William Fulbright Board of Foreign Scholarship, a fellowship with the Wexner Heritage Foundation, the recipient of the U.S. Secret Service's "Outstanding Dedication

and Contributions" award and the Connecticut Yankee Council of the Boys Scouts of America Distinguished Citizen Award.

Dr. Patrick Carrick

Dr. Patrick Carrick, previously served as a member of the Senior Executive Service, as Director, Homeland Security Advanced Research Projects Agency (HSARPA), Science and Technology Directorate, Department of Homeland Security. As the HSARPA Director, he guided the management of the national technology research and development investment for DHS. Carrick led five divisions, consisting of a staff of more than 200 scientists, engineers, and administrators in Washington, D.C. Each year, HSARPA selects, sponsors, and manages revolutionary research that impacts the future of the Homeland Security Enterprise. As HSARPA's principal scientific and technical adviser, he was the primary authority for the technical content of S&T's portfolio. He evaluated the directorates' entire technical research program to determine its adequacy and efficiency in meeting national and DHS objectives in core technical competency areas, and identified research gaps and analyzes advancements in a broad variety of scientific fields to provide advice on their impact on laboratory programs and objectives. He recommended new initiatives and adjustments to current programs required to meet current and future Homeland Security needs.

Carrick earned his Doctor of Philosophy degree in chemistry from Rice University in 1983 and was an assistant professor of physics at Mississippi State University, and Director of the Shared Laser Facility at the University of Oregon prior to joining the Department of Defense in 1989. He served for 10 years at Edwards Air Force, California becoming Chief of the Propellants Branch at the Air Force Research Laboratory Propulsion Directorate in 1994. He successfully led a team conducting cutting-edge scientific research and engineering. He also directed the High Energy Density Matter Program, which develops advanced rocket propellants and energetic materials. As a senior research physical scientist, he developed the first cryogenic solid hybrid rocket engine.

Carrick served for two years as the Air Force Program Element Monitor for Propulsion and Power Technologies and Deputy for Science and Technology Policy in the Office of the Deputy Assistant Secretary for Science, Technology and Engineering. He monitored and provided guidance for the $300 million science and technology investment in propulsion and power. He served on national steering committees for both rocket propulsion and turbine programs and was the lead editor and coordinator of the national report on hypersonic technology. Carrick also served as the Air Force representative to the Department of Defense Functional Integrated Process Team on Scientist and Engineer Career Field Management.

Prior to becoming part of HSARPA, Carrick was the Director of the Basic Science Program Office and the Acting Director of the Air Force Office of Scientific Research, in Arlington, Virginia where he guided the management of the entire basic research investment for the Air Force. He led a staff of 200 scientists, engineers and administrators in Arlington, VA., and foreign technology offices in London, Tokyo and Santiago, Chile. Dr. Carrick has published more than 25 articles in peer-reviewed professional journals.

Mark Dannels

Mark J. Dannels is the Sheriff of Cochise County, Arizona and is a 34-year law enforcement veteran. Sheriff Dannels holds a master's degree in Criminal Justice Management from Aspen University and is a Certified Public Manager accredited from Arizona State University. He is the current Chair of the Immigration and Border Committee with the National Sheriff's Association, a member of the Board of Directors for the Southwest Border Sheriff's

Coalition, and President of the Arizona Sheriff's Association. Sheriff Dannels has been recognized and awarded the Medal of Valor, Western States Sheriff of the Year, Sheriff's Medal, Deputy of the Year, Distinguished Service Award, Unit Citation Award, National Police Hall of Fame, Lifesaving Award, and dozens of community-service awards from service groups and governmental organizations.

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Carie Lemack

Carie Lemack is the co-founder and CEO of DreamUp, a provider of space-based education and media services. She is also the co-founder of Global Survivors Network, a global organization for victims of terror to speak out against terrorism and radicalization. Ms. Lemack has coordinated and inspired events in Jordan, Pakistan, and Indonesia, produced the award-winning documentary film *Killing in the Name*, spearheaded the website: www.globalsurvivors.org, and generated interest and coverage in media outlets worldwide. Ms. Lemack co-founded and led the non-profit, non-partisan organization Families of September 11th. She was previously an International Affairs Fellow at the Council on Foreign Relations and is currently a Senior Fellow at the Center for Cyber and Homeland Security at George Washington University

Jeffrey Miller

Jeffrey Miller is the Vice President of Security for the Kansas City Chiefs. Mr. Miller is responsible for developing and managing all safety and security plans and programs for all facets of club operations, including facility security for the training complex, Arrowhead Stadium, event day safety, vendor-operated security and traffic procedures, as well as team security. He also serves as the primary liaison between the club and the National Football League office with regards to all security matters. As Senior Vice President with MSA Security, he was involved in business development in all aspects of the company including Entertainment and Sports Venue Security, Crisis Communications, Explosive Detection K9, SmartTech, Investigations, Social Media Intelligence, Cyber Security and Executive Protection. As the CSO for the National Football League, he oversaw all facets of security for the league including all investigative programs and services, event security (including Super Bowl and International Series), Game Integrity Program, executive protection, the Stadium Security Program, the Fan Conduct Initiative and the Fair Competition Initiative. Additionally, he completed a 24-year career with the Pennsylvania State Police, retiring in 2008 as Commissioner, serving for nearly six years as the 18th Commissioner. As a cabinet secretary, he was responsible for implementing crime and crash reduction strategies, anti-terrorism efforts, and general policing practices including emergency response in all 67 counties in Pennsylvania. He holds an Associate Degree from the

University of South Florida, a Bachelor's Degree in Criminal Justice from Elizabethtown College, and a Master's Degree in Public Administration from the Pennsylvania State University. He is also a graduate of the 194th Session of the FBI National Academy in Quantico, Virginia, as well as the 27th Session of the FBI National Executive Institute. He is a Distinguished Alumnus of the Pennsylvania State University as well as an Alumni Fellow of the school

APPENDIX B – TASK STATEMENT

Secretary
U.S. Department of Homeland Security
Washington, DC 20528

Homeland Security

MEMORANDUM FOR: Judge William Webster
Chair, Homeland Security Advisory Council

FROM: Kirstjen M. Nielsen
Secretary

SUBJECT: **Emerging Technologies Subcommittee**

Pursuant to the September 18th, 2018 HSAC meeting, I instruct the Homeland Security Advisory Council (HSAC) to establish a new subcommittee titled the "Emerging Technologies Subcommittee" to provide recommendations regarding the following issues surrounding the increasing emergence of technological advancements:

It has long been a truism that today's innovations can become tomorrow's threats. But the current speed of technological change has resulted in a world in which emerging dangers are rapidly outpacing our defenses. New technologies- from artificial intelligence to unmanned aerial systems-have the potential to disrupt the status quo and fundamentally alter the security landscape.

DHS and its partners have a responsibility to look to the future in order to foresee technological advancements that might result in new threats and vulnerabilities. The Department must also put in place the right programs, policies, and procedures to mitigate potential dangers.

The Emerging Technologies Subcommittee will explore these challenges,and its mandate will include, but is not necessary limited to, the following:

1. Provide an assessment of the current state and perceived future advancements over the next 3-10 years of the most critical emerging technologies s that could pose a threat to the homeland security of the United States, such as but not limited to artificial intelligence and machine learning; quantum information science and quantum computing; 3-D printing; unmanned aerial and ground-based systems; synthetic biology and gene editing; and advanced robotics.

2. Analyze and provide insight into the ways in which such technologies could endanger the homeland, with a focus on those which have the highest likelihood of becoming a threat and those that pose the highest consequences to U.S. homeland security.

3. Provide recommendations to best mitigate the perceived deleterious impacts of the assessed technological advancements, including recommended DHS near and long-term actions. Provide an assessment on the perceived opportunities for DHS components to maximize the use of these new technological advancements to guard against emerging threats.

These recommendations are due to the full Council no later than 180 days from the date of the subcommittee's formation.

Thank you, in advance, for your work on these recommendations.

APPENDIX C – SUBJECT MATTER EXPERTS

JB Baron, MITRE, CUAS, Lead Systems Engineer, Next Generation UAS

Brien Beattie, Director, Foreign Investment Risk Management, DHS Office of Policy

Carlo Canetta, PhD, MITRE, Mechanical & Reliability Systems

Patrick Carrick, PhD, Chief Scientist, S&T

Susan Coller Monarez, PhD, DAS, PLCY

Heath Farris, PhD, MITRE, Gene Editing, Chief Scientist, Advanced Technology

John Felker, Director, National Cybersecurity and Communications Integration Center (NCCIC), DHS

Dr. Ron Ferguson, MITRE, Cognitive Science & AI

Stacy Fitzmaurice, Transportation Security Administration

Emily Frye, MITRE, Cybersecurity, Director, Cyber Integration

Gerry Gilbert, PhD, MITRE, Chief Scientist & Director, Quantum Systems

Brendan Groves, Department of Justice

David Harvey, PhD, MITRE, Homeland Security Research

Chuck Howell, MITRE, AI/ML, Chief Scientist, Dependable AI

James Murray, Director, United States Secret Service (USSS)

General Robert Newman, Operations Chief, Counter UAS, DHS S&T

Robert Perez, Deputy Commissioner, U.S. Customs and Border Protection (CBP)

John Pistol, former Administrator, Transportation Safety Administration (TSA)

Daniel Price, Principle Director, DHS Office of Policy

Gary Seffel, National Security Council, The White House

Angela Stubblefield, Federal Aviation Administration

Nitin Sydney, PhD, MITRE, Advanced Robotics, Group Leader

Gary Tomasulo, National Security Council, The White House

John Vehmeyer, Portfolio Manager, S&T, DHS

Yaakov Weinstein, PhD, MITRE, Emerging Technologies