EMERGEX VACCINES

The Case for a Universal Coronavirus Vaccine White Paper

Coronaviruses are a group of related, positive single-sense RNA viruses that were first discovered in the late 1960's and, therefore, offer a recent history of scientific study and human experience. While a lot remains unknown, and information is evolving regarding the present coronavirus pandemic (COVID-19), there is still significant scientific evidence that can be drawn upon that might better inform the path forward with respect to vaccine strategy and design.

The purpose of this White Paper is to consolidate these observations into a digestible format and to ensure key scientific facts are recounted as a means to further inform decision makers.

Emergex acknowledges that some of the observations have been made as a direct result of activities undertaken by its own teams as part of its commercial development programs for viral diseases. Emergex wishes to contribute these observations and conclusions to support or interpret other's studies in the spirit of advancing the global good.

Recommendations

1. A moratorium on all clinical trials for vaccines that induce antibody responses against the coronavirus spike protein is necessary until the vaccines are tested in murine and NHP models for antibody-dependent-enhancement (ADE) of lung pathology post-viral challenge.

2. Monoclonal antibodies that are humanized and contain human Fc receptor sequences should not be administered in human coronavirus therapeutic trials as they cannot be tested prior to administration for ADE in animal models, since ADE requires that the phylogenetic class of donor IgG antibodies be the same as that of the recipient's Fcγ-bearing cells.

3. Recombinant Complement Inhibitor (C1inh) should enter clinical trials to reduce complement-mediated lung inflammation in acute respiratory distress syndrome (ARDS) associated with COVID-19.

4. Immunotherapeutic and prophylactic vaccines that induce coronavirus cross-reactive CD8 T-cell immunity (and no antibody or CD4 response) should enter clinical trials as soon as possible (= Universal coronavirus vaccine).

Key Scientific Facts

1. Autopsy reports on patients who died of COVID-19 demonstrated that the virus primarily attacks the lungs with no evidence to support damage to any other organs.

2. Patients that recovered from COVID-19 showed no sign of virus in urine, blood, throat swab, or rectal swab. The virus is only found in nasopharyngeal swab, sputum and faeces.

3. Patients that recovered from COVID-19 had only minimal pro-inflammatory cytokines even whilst symptomatic.

4. Patients that recovered from COVID-19 had rapid elevation in CD8+ T cells just prior to resolution of symptoms.

5. Daily death rate data from China suggests the virus moves as a 4-week, Lorentz-shaped wave (± 25 days of peak) through a population (similar to "flu" 1951 epidemic of unknown aetiology). This contrasts with flat population transmission dynamics of flu pandemics that infect ~50% of a population over an extended period before reappearance in secondary or tertiary waves seasonally separated (1918 and 1957 and 1968 pandemics).

Conclusions

1. Pathology is consistent with COVID-19 being classified as respiratory tract-specific infection with only horizontal intra-organ spread.

2. Lack of viremia and a blood cytokine response in convalescent patients suggests that antibody and CD4 cellular immune responses play a small, if any, role in disease resolution.

3. Temporal correlation of a large cytotoxic T cell response with resolution of symptoms suggests the CD8 T cell immunity is important in resolution of disease without associated pathology.

4. Previous coronavirus pandemics may have occurred in the past and can provide clues as to the epidemiological behaviour of current COVID-19.

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Background History and Science of RNA Virus Diseases

Viral pandemics last much longer than the general conception about them. A newly introduced virus will circulate for many years until there are no more naïve hosts to allow its spread. Hence, pandemics are measured in decades, during which viruses compete for vulnerable hosts. The current coronavirus pandemic may follow a similar pattern. The 1918 “Spanish Flu Pandemic” is thought to have originated in Guangdong Province of Southern China between 1907 and 1917 and was then introduced to Europe via Chinese labourers digging the trenches there. The extensive death rate in Europe and in the US, which affected all ages of patients, suggested that these Western geographies contained “virgin soil” populations (those with little to no previous imprinted cellular immunity to this virus originating in Southern China). Importantly, Spanish Flu came in three pandemic waves (the summer of 1918, autumn of 1918, and winter of 1919), with the “third wave” associated with the highest death rate which then became endemic until 1949.

Cellular immunity to influenza does not include serotype recognition (i.e. H or N). Non-coding Open Reading Frame (ORF) length sequences of the negative strand of segment 8 (NEG8) - which is antisense - can be used to gain insight into cytotoxic T cell immunity to influenza which is required to clear the viral infection. The positive-sense transcription of segment 8 is NS1 and NS2 whereas the negative strand transcription is NEG8. T cells have only recognized three NEG8 lineages of influenza since 1918; one from 1918 to 1949, one from 1949 to 2009, and another from 2009 to the present – each transition corresponding to a pandemic at the time when the new NEG8 ORF appeared.

The H1N1 strain responsible for the 1918 pandemic (A/Brevig Mission/1/1918, NEG8 ORF167) and its descendants were the main, if not the only, circulating influenza viruses on the globe for the next 31 years… up until 1949 when they were abruptly and globally replaced by H1N1 A/Roma/1949 (NEG8 ORF216) at the time of the post-war pandemic. This new virus not only spread globally, but also displaced the previous Spanish Flu strain that had been present for the previous three decades. A/Roma/1949 and its descendants were eventually displaced 60 years later, in 2009, (Mexican/Swine flu) by H1N1 A/California/04/2009 (NEG8 ORF85). The speed in which A/California/04/2009 not only spread around the globe, but also displaced the previous circulating strain was astounding. Within a few months, it replaced all circulating strains of H1N1, including remnants of the refrigerator released version of A/Roma/1949 (H1N1 1976 Russian Flu (A/ New Jersey/1976). Direct viral descendants of these previous global occupants still circulate today in distinct geographic enclaves. The original Spanish Flu strain (H1N1, also now including H2N2 and H3N2 descendants) still circulates at low incidence. The present-day descendants are ~ 76% identical to the original 1918 strain and have attenuated in identity (virulence) in line with ‘Mueller’s Ratchet’ hypothesis that postulates that all high-mutation RNA viruses must rapidly lose virulence or go extinct.

Southern China is postulated to be the epicentre for the emergence of all pandemic influenza viruses. There has been a recycling of these viruses over the last 130 years in Southern China and, during the last 90 years, these viruses have recycled at least 4 times with the emergence of serotype variants of the H1 subtype (e.g. Asian Flu = H2N2 in 1957 and Hong Kong Flu = H3N2 in 1968 – both NEG8 ORF216). Co-circulating with the human-adapted influenza viruses in Southern China are also the nine highly pathogenic avian flu viruses (bird flu) (NEG8 ORF93) such as H5N1, H7N9, etc., as well as other severe acute respiratory viruses, such as severe acute respiratory syndrome (SARS). The SARS outbreak in 2003, like 1918 Spanish Flu, was traced to Guangdong Province of Southern China to restaurant workers handling wild animals as exotic food, with a bat coronavirus being the probable source.

The present coronavirus pandemic (COVID-19) probably emerged from this same Southern China breeding ground of viruses, with the current postulated source of the zoonosis being live animal markets, similar to the SARS origin nearly 2000 km distant. The 2013 bat coronavirus (Pu’er City isolate -RaTG13) from Southern China has been proposed as a potential lineage example of human COVID-19. The RaTG13 bat isolate was collected in 2013, and between 2013 and 2017 scientific expeditions to bat caves led by EcoHealth Alliance USA and Wuhan Institute China collected ~500 coronaviruses.

Emergex has performed an analysis of the 238 reported COVID-19 isolates deposited in international databases that indicates that they can also be classified via *a negative strand ORF length (present in negative strand of nsp3) which is essentially identical between all of the isolates reported to date*. The only other coronaviruses to have an identical ORF length are three bat isolates: the 2013 RaTG13 isolate, and the 2015 and 2017 isolates (RaTG13 – Wuhan Institute of Virology, CoVZXC21 and CoVZC45 - Institute of Military Medicine Nanjing). Interestingly, the SARS virus from 2013 does not share the ORF identity of COVID-19.

RNA viruses circulate as a "cloud "of variants as RNA replication does not have the editing mechanism inherent in DNA replication. These variants are called "quasi-species". There is evidence that evolutionary selection targets RNA virus quasi-species populations, rather than targeting individual variants, and it is the cooperative interactions between variants that influences virus pathogenesis. The estimated monoclonal antibody escape rate for the RNA measles virus was measured at a mutation rate of 9×10^{-5} per base per replication, and a genomic mutation rate of 1.43 per replication. During a natural infection, the generation of the variants is in direct equilibrium with the recognition of these variants by the adaptive immune system, thereby giving rise to a polyclonal antibody response that matches the population. *There is a disconnect between infection and disease onset for RNA viruses: RNA viruses only cause disease once a critical number of variants is established.* They cause disease by group action and the number of variants required to cause disease is called the "bottleneck size". The time that it takes to reach the critical variant number will determine the incubation time prior to clinical disease. Since each founder population is statistically different for every infection, this difference will give rise to a large variation/dispersion in incubation times. For COVID-19, the incubation times have been estimated to range from several days to weeks.

Coronaviruses are positive single-sense RNA viruses and the only RNA viruses that have an RNA-dependent RNA proofreading machine. The enzyme, ExoN, is coded on nsp14 and generated from the polyprotein. Nsp14 exonuclease mutant of SARS-CoV has a mutation frequency 21-fold increased during replication in culture. Mutations in nsp14 therefore decrease fidelity and increase quasi-species diversity on viral replication, pathogenesis and evolution. *Emergex has found that COVID-19 contains 28 mutations in nsp14 compared to SARS, suggesting that COVID-19 has reverted to a full quasi-species virus.* Loss of fidelity will also result in increased mutational frequency of other COVID-19 proteins, and *Emergex have found that the spike protein of COVID-19 differs by 309aa (24aa changes per every 100 aa) compared to SARS*.

Reversion to quasi-species characteristics also has implications for sequence analysis and design of RNA and DNA vaccines, since database sequences that are usually applied for the design of these vaccines now only represent consensual sequences, and therefore not a unique structure that actually exists amongst the quasi-species (i.e. they only predict an average of averages). Full quasi-species viruses such as influenza or dengue always require a "start-over" from a small donor inoculum to a recipient upon infection in order to prevent the build-up of lethal mutations that could lead to extinction of the virus (Mueller's Ratchet hypothesis). **Consequently**, each dengue mosquito injects a unique variant population (the same conclusion for aerosol influenza transmission) which then expands in the recipient – so would be the case for COVID-19 if it has lost ExoN activity. Antibody escape mutants would make spike vaccines problematic.

RNA virus quasi-species present unique problems to vaccine development. It follows that, **historically, the only vaccines that have ever worked against RNA viruses have been empirical, isolated, live attenuated vaccines** (measles, yellow fever, poliomyelitis (polio), rotavirus, etc.). These vaccines give lifetime (~25 years) T cell immune memory responses. Four inactivated versions of the live attenuated viral vaccines have been on the market: [1] inactivated poliomyelitis which was eventually removed because it was discovered that vaccinated individuals still spread the live virus upon infection; [2] rabies, which does not protect, but simply eases post-infection therapy; [3] hepatitis A, which has never been shown to provide protection (it results in high neutralizing antibody titres as a surrogate marker) and [4] rotavirus which is only required to give a very limited period of protection in children in contrast to lifetime immunity.

Failures of Inactivated and Recombinant Live Attenuated Vaccines

It is, therefore, important to understand the mechanism of action of the live attenuated vaccines and how they are able to provide protection. Next-generation deep sequencing of *the vaccine isolates confirmed these live attenuated vaccine preparations contain a limited number of variants, if not just a single variant.* This variant restriction has occurred because of extreme growth pressure in the cells in which the isolates are manufactured, such that most of randomly formed variants do not form functional virions for re-infection of uninfected cells, a process that is required to grow up a vaccine from a small inoculum. For yellow fever vaccine, the single variant found in the vaccine was not found in wild type yellow fever virus strain. It follows that, at the time of vaccination, the virus no longer has the growth restraints of the cell line of manufacture and thus it propagates *in vivo* and generates quasi-species by mutation – which now are also contained in the wild-type virus. The vaccine is therefore generated *in vivo* post-vaccination. Poliomyelitis provided a more dramatic example when the shed virus from children vaccinated with live poliomyelitis vaccine could cause poliomyelitis. That is, a population of variants could emerge by chance from the initial immunizing inoculum that could have neural tropism. In general, *only subclinical disease is caused by the live attenuated vaccines, since the time to generate a sufficient number of variants from the very small donor populations is usually longer than the adaptive immune system requires to mount a T cell response and eliminate cells infected by the vaccine.* Natural IgM (nIgM), that does not engage with Fc receptors, is also important in the immunization of a naïve individual and neutralizing IgG antibody does not appear until many weeks after either immunization or a natural infection.

Emergex is only aware of one entity attempting to produce a live attenuated COVID-19 vaccine. The method of attenuation is to make a synthetic virus using non-optimal codons and thus slowing viral proliferation. However, it is not clear how manufacturing purity can be maintained, due to a lack of selective variant pressure by a cell line such that new mutations rapidly accumulate, regenerating the wild type. Furthermore, not a single DNA, RNA, protein subunit, VLP, recombinant virus vaccine, inactivated attenuated vaccine, etc. has ever been shown to be effective for RNA viruses and attempts at a SARS vaccine were unsuccessful when the antibody component caused lung problems in experimental animals.

The failure of inactivated attenuated viral vaccines to provide protection against RNA viruses has been extensively studied. A virus must grow inside a cell in order to generate both dominant and subdominant Class I peptides that can be transferred and then expressed by antigen-presenting cells (APC) to generate T cells that will recognize and kill the viral infected cells, expressing these same peptides. Inactivated attenuated vaccines generate only a limited dominant T cell response via a process called “cross-presentation”. This process also depends on the mechanism of inactivation, with large differences being seen between heat and chemical inactivation.

Cross-presentation is a Class II-mediated process in which exogenous cellular proteins (after uptake of the inactivated virions) are cleaved with proteases in the cytoplasm in order to be presented by antigen presenting cells (APC) to CD4 naive T cells. These activated CD4 T cells can assist in antibody formation (B cells) and CD8 T cell activation via CD4 help. However, the only Class I peptides that can be formed in cross-presentation are Class I peptides that are “nested” within the larger Class II peptides. This process severely restricts the repertoire of expressed Class I MHC antigens. Further, since the virus is not growing in an APC that has taken up the inactivated virion, peptides can only be generated relative to the abundance of proteins found on a final intact virion. For example, structural proteins are in far greater molar abundance than non-structural internal proteins in an intact virion. The temporal generation of T cell receptor class I epitopes is also important, with *early viral antigens thought more important than late antigens in generating an effective T cell response. This temporal information can only be obtained in cells in which the virus is actively replicating, and the information is lost when the virus is dead.*

Infection of cells with living viruses also significantly changes the mass action dynamics of Class I peptide presentation. The main six Class I alleles present on a human cell (two each of HLA A, B, C)

are presenting the self-peptide repertoire of the cell, estimated at 2 billion peptides. If viral peptides had to compete with this number of self-peptides, they would have only a very low statistical chance of binding to the 100,000 HLA Class I molecules expressed on a single cell as peptide turnover occurs. The processing of viral Class I peptides from viral proteins synthesized within a cell comes from a separate pathway called the "immuno-ribosome". This endoplasmic pathway (in contrast to the Class II cytoplasmic pathway) does not compete with the self-peptide pool.

Emergex have found experimentally that, upon viral infection, most of the self-peptides already expressed on the cell surface get rapidly replaced by viral peptides. This replacement process is critical since **the virally-infected cells now contain multiple viral targets in high abundance such that they can be targeted by an extensive repertoire of T cells of many binding affinities (the focus of Emergex's approach to treating the viral disease)**. This contrasts with the high affinity dominant T cells that will bind to low abundance, but high affinity viral derived peptides amongst the overwhelmingly dominant self-peptide repertoire.

Recombinant vaccines can only generate T cell responses against peptides present in the protein construct that was actually included in the vaccine (plug-and-play concept). All the other T cell responses are off-target to the host virus: the responses will be against the carrier virus. For example, vaccine constructs made from adenovirus or VSV backgrounds can only potentially generate T cells against peptide determinants of the single surface glycoproteins that are inserted. These vaccines are designed to generate antibody responses against the viral target protein. Further, it is only the internal, non-structural proteins of viruses that give effective T cell responses as these seem to be the predominant source of MHC expressed peptides. It follows that a recombinant vaccine will not produce a cellular immune response against the target virus, just the host virus. Examples of recombinant vaccines in development include chikungunya proteins in measles, influenza proteins in adenovirus, HIV proteins in canary pox, Ebola proteins in VSV, Ebola proteins in adenovirus, etc. Three of these approaches have had total failures in the clinic, with Ebola protein in VSV only being used for ring vaccination studies (if given within 10 days of exposure) in which the mechanism of action is unknown. One recombinant live attenuated vaccine included the insertion of dengue structural protein genes into a live yellow fever attenuated virus. That stratagem was the basis for Sanofi's *Dengvaxia*, the only dengue vaccine that has progressed through the clinic to commercial use, but it was subsequently withdrawn or severely restricted in use due to an antibody-dependent-enhancement (ADE) clinical reaction, a worsening of the infection/disease. Learning the lesson from these failures is critical for the development of an effective, safe coronavirus vaccine.

Augmentation of RNA Viral Disease by Vaccination:

Contra-Lessons for the Development of a SARS-2 Vaccine

It is well established in the immunology/vaccine literature that most, if not all, RNA viruses are capable of using IgG (or the B cell receptor (BCR)) as a pathogenic factor. At least three mechanisms have been established:

1. Extrinsic antibody-dependent-enhancement (ADE) is a process in which IgG binds to circulating free virions and increases the number of infected cells by using the IgG Fc to infect cells that have Fc receptors.

2. Intrinsic ADE is a process whereby IgG immune complexes formed of virions bind to monocytes and macrophages and, via cytokine and other factors, suppress innate immunity and host defences, thus leading to increased infectious output by previously infected cells.

3. B-cells with Ig receptors that are against RNA viruses (i.e. the source of the IgG) can be targeted by the virus, thereby killing protective viral-specific B cells. Infection and killing of a fraction of the rare, antigen-specific B cells impairs the kinetics of the memory response and

confers an advantage to a virus that has replication cycles measured in hours. The ability of the coronavirus to eliminate the initial wave of the very B cells capable of counteracting the infection is an efficient means of ensuring an unfortunate window for virus replication and horizontal transmission.

The soluble IgG-mediated ADE requires the presence of IgG simultaneously with viremia. In a natural infection, this context is avoided. The initial humoral response is natural IgM (nIgM), which joins forces with the innate immune system to limit the expansion of the RNA cloud until naive T cells can be stimulated to proliferate, differentiate into CD8 effector T cells and then kill the infected cells. IgG antibodies normally occur after a primary infection has cleared. The peak of neutralizing antibody in a naïve population is around Day 35, with sera conversion happening around Day 20. For recall responses, the timing is shorter (25 and 12 days respectively). For dengue, ADE leading to dengue haemorrhagic fever (DHF) has never been reported for a primary infection but occurs in secondary infections by a different dengue viral strain. Dengue is considered to consist of 4 separate viral strains, in which the surface protein differs, and the internal proteins have significant sequence homology. The ADE occurring on the secondary dengue infection has been shown to be IgG antibody-mediated. The phenomenon has been termed “original antigenic sin” and it punishes the ‘sinners’ (the infected) severely.

It follows that immunization of naïve individuals by a vaccine that generates an IgG response against dengue is a surrogate for the first natural infection such that, upon a subsequent natural infection, the ADE process occurs, and the sinners suffer. This outcome was the experience for the Sanofi *Dengvaxia* vaccine leading to DHF in primary infections. Historically, this outcome might have been predicted. In the 1960s, antibody-stimulating vaccines (using formalin inactivated viruses) were tested as vaccines against measles and respiratory syncytial virus (RSV). Individuals vaccinated against measles were not protected and, upon subsequent infection, had a different clinical picture which was subsequently named ‘atypical measles’. Individuals up to 17 years following this vaccine trial could still develop atypical measles. For children given inactivated RSV vaccine, 80% of the vaccines needed hospitalization whereas only 5% of the RSV-infected children in the control vaccine group required hospital admission. During the 2009 influenza pandemic (and the 1957 pandemic), the main cause of death was immune complex mediated lung pathology, an outcome suggesting that those individuals with recent prior influenza of another virus subtype, or recent vaccination, were already at risk.

In the 2003 SARS epidemic, the main cause of mortality was acute respiratory distress syndrome (ARDS). This clinical result occurred in two populations, both simultaneously with IgG sera conversion:

- The first population of ARDS occurred in individuals at around Day 12 coincident with IgG sera conversion.
- The second population occurred in separate individuals at around Day 20, again coincident with IgG sera conversion.

Age incidence studies for SARS indicated that children younger than 12 years of age were largely unaffected. This feature suggested that prior alternative coronavirus infection might predispose persons to ADE in the older population in contrast to providing protective immunity (heterologous immunity). The first population of ARDS patients would be consistent with a group having prior exposure due to the rapid IgG recall, compared to the second group that presumably would have been naïve individuals. The ARDS in the deceased SARS patients was subsequently demonstrated to be antibody-mediated. The SARS pattern of clinical response is suggestive of dengue, in which prior immunity to one strain predisposes the pathology when infected with a second strain – a clear warning to Public Health authorities of a likely second wave of COVID-19 disease which will be more lethal.

Using extensive sequence analysis, Emergex have found that the 2003 SARS and the current COVID-19 can be considered related viral strains (three spike protein types) and now other authors have already started using the nomenclature SARS 1 and SARS 2. In the current COVID-19 pandemic,

individuals younger than 20 years old appear to have minimal disease, with the majority of morbidity occurring in the over 70s age range. *This data suggests that prior exposure (unknown previous immune imprinting) may lead to a negative clinical outcome.* A cohort in which pathology is predominantly evident in over 70s could indicate that a previous coronavirus covertly circulated circa 1950s to which these persons had exposure. Such an event could have been the 1951 Flu epidemic that, in some localities, had weekly death rates that was ~40% higher than the peak of the 1918-1919 pandemic. As death rates were classified for "pneumonia and flu", this epidemic could have had an alternative infectious source, as influenza diagnosis was still in its infancy at this time. Importantly, this conclusion has been seen with other viral infections where there is an association of past dengue fever epidemics with the parallel risk of Zika microcephaly. In this case, a unique birth cohort of women exposed to a previous dengue epidemic had risk of Congenital Zika syndrome 7-12 years after exposure.

Other clinical data from previous experience with SARS supports the presence of an ADE mechanism in the lung pathology of that disease. In summary:

1. Patients with SARS-CoV, Mer-CoV, H5N1, H7N9, etc. all display characteristics of acute lung injury (ALI).
2. Patients who die of SARS develop severe ARDS.
3. 80% of patients who present with ALI develop ARDS, parallel with IgG seroconversion.
4. Anti-spike neutralizing antibody (NAb) responses against SARS spike surface glycoprotein developed faster in deceased patients: 14.7 days for those who died compared to 20 days for recovered patients.
5. NAb titres were found to be significantly higher in deceased patients compared to recovered patients.

Data from attempts to make a vaccine for SARS has shown, to date, that there is no protective effect against pulmonary immunopathology mediated by full-length S-protein based vaccines in SARS-infected nonhuman primates. Similar results were also reported for mice and ferrets. Specific reduction in viral clearance, nor protection against lethal challenge, correlate with protection against lung pathology.

In summary:

1. **Multiple vaccine platforms and viral infections induce SARS-specific immune memory that enhances lung inflammation following homologous viral challenge in mice, ferrets and nonhuman primates.**
2. **Both prior vaccination and passive administration of anti-S-IgG leads to massive accumulation of monocytes/macrophages in lungs within 2 days post-infection.**

Conclusions

The observation that coronaviruses like SARS and COVID-19 may be exhibiting a disease dynamic and pathology similar to dengue, both in terms of heterologous immunity and strain dynamics, is critical for vaccine development. The current approaches to dengue (and other universal vaccines against RNA viruses) is based on enhancing the background T cell response. These approaches, however, need to be selective since generating a vaccine response against "both" IgG and T cells (or just IgG) could lead to significant immunopathology.

T cell immunity within the context of heterologous immunity is powerful since the evolution of T memory cells, upon repeat infection with different strains of coronaviruses, leads to cross-reactive immunity in contrast to the humoral immunity which stays "fixed" to the initial strain characteristics (original antigenic sin).

From a scientific standpoint, **a vaccine that generates cross-reactive T cell immunity to coronavirus and no humoral component should be the focus of accelerated development. This is the objective that Emergex has focused its efforts upon, enabling such a vaccine strategy.**

Emergex Vaccines Holding Ltd

Emergex Vaccines Holding Limited ("Emergex") is a private company based in Abingdon, Oxfordshire in the UK. Its primary focus is on the development of medicines for the prevention or reduction of viral-related illness as well as some intracellular bacteria-related diseases. Emergex has termed its therapeutic agents **'set-point vaccines'**. The Company aims to develop set-point vaccines that are population-based, inexpensive and can be delivered relatively quickly; thus, they will be capable of better intervening effectively in infectious outbreaks.

Emergex's set-point vaccines enhance the body's natural immune response. They do not include any DNA or RNA and the components are entirely (100%) synthetic.

When a virus infects a cell, that infected cell reactively produces a peptide 'code' that is then expressed on the surface of the infected cell. This encrypted code is presented on the surface of the infected cell by HLA Class I molecules. In healthy uninfected cells, this internal code presented on the cell surface provides for recognition of "self" and prevents the immune system from eliminating that cell as foreign. For those infected cells, the viral 'code' however is recognized as foreign (not self) and the human immune system will kill and eliminate the infected cell via cytotoxic T lymphocytes (CTLs) called CD8+ T cells.

Instruction to the immune system regarding the viral antigens occurs via physical transfer of the viral peptides from the infected cell to the immune system. Emergex uses state-of-the art technologies to determine the exact antigens produced by a virus and then to deliver those antigens to the immune system via dendritic cells for CD8+ T cell programming….essentially programming and priming the immune system to perform its kill-and-clear functions.

CD8+ T cell programming prior to a viral infection changes the immune 'setpoint', such that upon first exposure to the infecting virus, the immune system is able to respond much faster. The increased speed of response limits disease progress, but still allows for primary infection, which drives lasting and long-term immunological memory (i.e. a consequence of natural infection and natural immunity). A proprietary gold nanoparticle system technology is used as the delivery system for transferring the peptide antigens to the correct immune system components.

Emergex has a growing pipeline of vaccine candidates including a Coronavirus vaccine based on the post-convalescent T cell response from patients from the 2003 SARS epidemic and then modified to be cross-reactive with COVID-19. An MHC expression ligandome library is currently being determined experimentally that will define the "peptide" Class I expression library on human lung cells to insure a more precise second-generation construct.

The most advanced development programme focuses on the flavivirus family with an initial target indication of Dengue Fever. It is expected that immune 'setpoint' pre-programming will occur against other related flaviviruses, such as the Zika and Yellow Fever viruses, resulting in a single vaccine that targets multiple viruses within a single genetically conserved family of viruses. Applying the same principles, the Company also has programmes in development for a universal influenza (including pandemic flu) vaccine and for a universal filovirus (Ebola and Marburg, all strains) vaccine.

Discovery programmes include a Yellow Fever Booster vaccine within Emergex's viral disease programmes and a vaccine against *Francisella tularensis* within Emergex's intracellular bacterial disease programmes. *Francisella tularensis* is the causative agent of tularemia, a form of pneumonia which is often lethal without treatment and is classified by the US government as a Tier 1 potential bioterrorist agent.

In June 2019, Emergex signed a development agreement with A*Star's Institute of Molecular and Cell Biology (IMCB) in Singapore to develop a vaccine for the highly contagious Hand, Foot & Mouth Disease. The agreement targets the development of a cross-therapeutic vaccine to protect against the group of viruses which are associated with HFMD, including enterovirus 71 (EV71) and coxsackievirus A16. EV71, a non-polio enterovirus, has recently been associated with paediatric acute flaccid myelitis (AFM) in the USA. AFM is a rare but serious condition. It affects the nervous system, specifically the area of the spinal cord called grey matter, which causes the muscles and reflexes in the body to become weak.

In 2019, Emergex announced that it had successfully secured a research and development (R&D) facility at Milton Park, Oxfordshire. This facility extends the Company's overall internal control of all its preclinical vaccine development programs. Key personnel at the site, who are highly experienced in the design, production and development of nanoparticles also joined Emergex, enhancing its internal resource of skills and expertise in vaccine development. In-house cGMP manufacturing facilities are also being established at Milton Park.

Precis of Emergex Technology and Its Advantages

Emergex combines validated technologies, together with the very latest scientific insights to develop its set point vaccines:

- Emergex has successfully generated a 1st generation human specific MHC-Class I CD8 peptide ligandome library for Dengue, flu, Zika, Hepatitis B and Francisella tularensis for the most commonly occurring human alleles. The library contains encrypted peptide data to instruct the immune system to alter the initial 'setpoint' of response on first exposure and potentially to reduce disease severity [but still allow natural immunity to provide long term protection]. Emergex's vaccines are self-adjuvant and also potentially limit or eliminate allergic, autoimmune or antibody mediated side effects of traditional vaccines.
- Where practical, blood samples of individuals with natural/acquired immunity to the pathogen are used to validate those peptide codes which were used to generate immunity in a natural infection.
- These validated peptides are combined with an extremely small (quantum) gold nanoparticle that can directly deliver to the naive immune system and then can program the immune system to eliminate pathogen-infected cells upon subsequent exposure and infection. It is the combination of these technologies that produces a set-point vaccine, capable of delivering the right peptides to the right place, in order to produce a strong T-cell immune response that will target and kill infected cells.
- Emergex's vaccines are suited to be administered by novel microneedles technologies.

Emergex's vaccines are advantageous in numerous ways:

1. They should reduce viral disease to subclinical by priming a T-cell mediated immune response.
2. Emergex's vaccines are self-adjuvant and limit or eliminate allergic, autoimmune or antibody mediated side effects of traditional vaccines. Emergex vaccines do not induce an antibody response [such as ADE].
3. They are 100% synthetic and also contain no RNA and DNA - do not use inactivated or live attenuated pathogens and therefore should be inherently safer to develop and use.
4. They replicate the cellular immune responses to highly conserved recognition elements of the pathogen often present in internal proteins in which selective pressure for mutation is minimal. An advantage of this approach is that these internal components are conserved within viral strains of the same virus superfamily, making feasible a broad/universal vaccine to tackle highly mutagenic viruses such as seasonal flu or COVID-19.
5. The current Emergex vaccines are designed to be delivered by novel microneedle technologies (for example a skin patch) which means that there is less need for primary healthcare providers

to administer the vaccines, no need for travel to central health clinics, hence improved compliance – a major challenge to providing vaccination in crisis conditions or in developing-world settings. Future planned dry patch vaccines are stable at room temperature, thereby avoiding the need for refrigeration, which enables easy transportation to the crisis locations and to remote parts of the world where they are most needed.

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Coronaviruses, SARS, COVID 19

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