1 SCIENTIFIC ABSTRACT

Hepatitis C is a major cause of morbidity and mortality worldwide. An estimated 170 million individuals are infected with hepatitis C virus (HCV) worldwide, with nearly 4 million people chronically infected in the U.S. and up to 9 million people chronically infected in Europe. Of those exposed to HCV, 80% become chronically infected, and at least 30% of carriers develop chronic liver disease, including cirrhosis and hepatocellular carcinoma. The current standard of care for patients in developed countries with chronic HCV infection, interferon α (IFN-α) and ribavirin, has demonstrated differential effectiveness amongst the most prevalent HCV genotypes 1-3. In addition, the toxicity and tolerability profiles of IFN-α and ribavirin limit their use in HCV treatment. Thus, there is a continued need for effective new therapies.

HCV has developed mechanisms to evade immune elimination, thereby allowing it to persist in the liver in the majority of infected individuals. These evasion mechanisms are not absolute and therefore could be reversed or circumvented by an appropriate immunotherapeutic strategy. Anza Therapeutics, Inc. (Anza) has developed an immunotherapy strategy based upon a live-attenuated *Listeria monocytogenes* strain (*Lm* Δ*actA*/Δ*inlB*) that can potentially enable the host's immune system to eliminate the virus in chronically infected individuals via stimulation of an innate and adaptive immune response.

A significant barrier to the development of an HCV therapeutic vaccine is that HCV is a highly diverse virus. To address this virus sequence diversity, the ANZ-521 investigational agent encodes a consensus sequence corresponding to portions of the HCV NS5B and NS3 proteins that also includes the incorporation of directed mutations to abrogate any potential activity of the fusion protein. Use of a consensus sequence reduces the number of amino acid differences between the antigen and circulating strains and maximizes the number of epitopes shared by the vaccine sequence and any individual circulating strain, thus increasing the likelihood that vaccine-induced immune responses will be reactive against the specific virus infecting a given individual. The HCV antigen-encoding sequence was synthesized to utilize optimal transcription codons for expression in *Lm*. In addition, the HCV antigen expression cassette in ANZ-521 exclusively utilizes *Lm* transcription, translation, and secretion machinery, and it does not contain any mammalian expression elements (such as promoter or terminator regions characteristic of mammalian expression systems). Therefore, unlike plasmid DNA- or viral-based vectors that must utilize the mammalian host cell machinery to express a designated

gene of interest, and where gene transfer is a prerequisite for gene expression, *Lm* is a "self-contained" free-living organism. Within infected cells of the immunized host, the prokaryotic expression machinery of ANZ-521 is utilized exclusively to synthesize the HCV NS5B-NS3 fusion protein within the bacterium, which is subsequently secreted into the cytoplasm of the infected host cell for antigen processing. Thus, the attenuated *Lm* organism (ANZ-521) that will be administered to human subjects in the proposed Phase 1 trial contains recombinant DNA having prokaryotic regulatory elements that are not functional in mammalian cells.

The ANZ-521 investigational drug product consists of the recombinant live-attenuated *Lm* Δ*actA*/ΔinlB/NS5B-NS3 strain BH2064 that encodes the HCV NS5B-NS3 consensus sequence antigen that has been formulated in a phosphate buffered saline containing glycerol. The ANZ-521 investigational drug product is stored frozen at or below –60°C. ANZ-521 has the same genetic background as two other investigational agents, known as ANZ-100 and CRS-207, the latter of which was reviewed by the full RAC committee on June 21, 2007 (RAC Protocol 0704-853). The only difference is the replacement of the Mesothelin coding sequence in CRS-207 with the HCV NS5B-NS3 coding sequence in ANZ-521. Preclinical safety studies have shown that the virulence, biodistribution, and acute toxicity of ANZ-521 is comparable to the ANZ-100 and CRS-207 *Lm* strains. In clinical trials with ANZ-100 (completed) and CRS-207 (ongoing), no clinically significant safety signals or liver toxicities related to investigational agent have been observed at doses up to 1×10^9 cfu. Consistent with preclinical findings, the clinical observations indicate that the insertion of an antigen-encoding expression cassette does not significantly alter the safety profile.

The proposed Phase 1 clinical study with ANZ-521 is a randomized, placebo-controlled, multiple-dose escalation followed by dose expansion study in treatment-naïve genotype 1 hepatitis C patients with compensated liver disease. It will consist of a dose escalation stage (Part A), a dose expansion stage (Part B), and an open-label roll-over stage for placebo subjects (Part C). The proposed doses to be evaluated are 1×10^7, 1×10^8, and 1×10^9 cfu. The initial proposed dose is 100-fold below the highest dose evaluated with similarly attenuated *Lm* agents (ANZ-100 and CRS-207) already evaluated in humans and also chimpanzees chronically infected with HCV. The highest proposed dose is equivalent to doses well-tolerated by humans (CRS-207) and also chimpanzees chronically infected with HCV (ANZ-100).

The proposed clinical trial is designed to evaluate ANZ-521 in treatment-naïve genotype 1 patients who are candidates for treatment but are intolerant to or otherwise

refuse standard of care (i.e., interferon-α and ribavirin). Patients who fail to respond to standard of care have been reported to be less responsive in general to immunologically-targeted agents, such as ANZ-521. Thus, patients who have failed standard of care would have a lower likelihood of benefiting from ANZ-521 therapy, which is designed to stimulate the production of local interferon-α and other cytokines and chemokines in the liver, as well as to recruit and activate NK cells and CD4+ and CD8+ T cells. At the same time, the risks of administering ANZ-521 to treatment-naïve and treatment failure patients are not expected to be different. Therefore, the risk-benefit analysis favors enrollment of treatment-naïve patients who are more likely to benefit.

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