



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

MEMORANDUM

Food and Drug Administration

DATE: July 12, 2012

FROM: Haiyan Qin

TO: Katherine Berkhousen
Richard Daemer

Through: Marian Major
Robin Levis
Sara Gagneten

SUBJECT: STN 125428/0 Hepatitis B Vaccine (Recombinant) [HEPLISAV™]

SPONSOR: Dynavax Technologies Corporation

This review memo pertains only to the preclinical immunology data (4.2.1) contained within this original BLA.

Introduction:

HEPLISAV™ is a new hepatitis B vaccine utilizing an immunostimulatory thiolated CpG oligonucleotide as the adjuvant. The immunogenic component, hepatitis B surface antigen (HBsAg), subtype adw, is produced in the yeast strain *Hansenula polymorpha* using recombinant technology. The HBsAg (adw subtype) is manufactured by the Rhein Biotech GmbH, Dusseldorf, Germany, a wholly owned subsidiary of Dynavax. In previous phase I and II clinical trials the HBsAg (adr subtype) component was made by the (b) (4) (b) (4) using a similar protocol as the present manufacturer. HBsAg is a lipoprotein particle of approximately (b) (4) in size, the protein component consisting of a single polypeptide chain containing (b) (4) amino acids with a theoretical molecular weight of (b) (4). The 1018 ISS Adjuvant is a 22-mer immunostimulatory phosphorothioate oligonucleotide with a molecular mass of (b) (4) that is produced by (b) (4). HBsAg Drug Substance is formulated with 1018 ISS Adjuvant to produce HEPLISAV Drug Product.

HEPLISAV Drug Product is a sterile, liquid dosage form that is administered as an intramuscular injection. HEPLISAV Drug Product is formulated as 6000 mcg/mL 1018 ISS Adjuvant and 40 mcg/mL HBsAg Drug Substance in 8 mM sodium phosphate, 154 mM sodium chloride, 0.01% w/w polysorbate 80, pH 7.0 buffer. The finished vial (0.7 mL) contains 4200 mcg of 1018 ISS Adjuvant and 28 mcg of HBsAg Drug Substance of which an administered dose of 0.5mL contains 3000 mcg of 1018 ISS Adjuvant and 20 mcg of HBsAg

Drug Substance. The intended clinical use is as a prophylactic vaccine for adults against infection with hepatitis B virus. The dosing regimen is for each adult to receive two intramuscular injections from a healthcare provider of a 0.5 mL dose, the first at an elected date and the second one month later.

Summary:

The preclinical immunology data contained in this original BLA demonstrated that the HBsAg + 1018 ISS (3000 ug) was highly immunogenic and superior to Engerix-B in terms of antibody titers in a number of studies. Further, co-administration of HBsAg + 1018 ISS did not induce substantial anti-dsDNA antibodies, nor did it induce significant changes in the serum chemistry or hematology in most of the study baboons. Therefore, the preclinical studies supported full clinical trials to test safety, immunogenicity and efficacy of the vaccine in humans. I do not have any major concerns to the preclinical immunology studies of this submission.

The nonclinical program included studies to assess the pharmacological properties and toxicity profile of both HBsAg Drug Substance plus 1018 ISS Adjuvant, or of 1018 ISS Adjuvant alone. Pharmacology studies established the effectiveness and dose response of 1018 ISS Adjuvant as an adjuvant for HBsAg Drug Substance. No severe toxicity was observed in studies of the HBsAg Drug Substance combined with 1018 ISS Adjuvant and all effects were consistent with known class effects. A multi-generation reproductive toxicity using a 25-fold excess relative to the human dose for HBsAg Drug Substance and a 200-fold excess relative to the human dose for 1018 ISS Adjuvant did not identify adverse effects on any of the parameters evaluated. Studies of 1018 ISS Adjuvant alone using a 272-fold excess to the human dose demonstrated rapid elimination of 1018 ISS Adjuvant from the plasma and produced expected class specific toxicities.

Dynavax is currently submitting this application for consideration of US licensure for HEPLISAV™ with the following proposed indication: Prevention of infection caused by all known subtypes of hepatitis B virus in adults age 18 through 70 years. The immunization schedule consists of 2 doses given 1 month apart.

Comments to the Sponsor

The following question was submitted to the sponsor on July 26, 2012.

On page 3, please clarify whether the HBsAg used for (b) (4) is the same as the one for immunization of the mice. If not, what is the source for HBsAg used to (b) (4) ?

The sponsor responded to CBER's above inquiry on Aug 9 2012 (amend. #003). The sponsor confirmed that the (b) (4) antigen used for the mouse antibody (b) (4) was (b) (4) and is the same as the one for immunization of the mice. Based on this piece of information, the sponsor should keep in mind that the reported antibody responses elicited by immunization with HBsAg, HBsAg & (b) (4), and HBsAg & 1018 ISS could be higher than the actual value, even though this is not a major issue at this stage of the vaccine development.

The review pertaining to the preclinical immunology data of this submission is documented below:

Study No. 99-0086

Purpose of experiment:

To determine the immune responses of mice immunized with 1018 ISS mixed with recombinant yeast derived HBV antigen; to determine if mice immunized with HBV + 1018 ISS generate antibodies to ssDNA and dsDNA.

Summary:

Mice that were immunized twice with HBsAg alone or HBsAg mixed with (b) (4) generate a Th2 profile of antibody responses similar to the response elicited by Engerix vaccine (high IgG1 and lower IgG2a). In contrast, mice immunized twice with HBsAg mixed with 1018 ISS generate a Th1 profile of antibody response (high IgG2a and low IgG1). 1018 ISS shifts the anti-HBsAg immune responses from Th2 to Th1 in mice injected with recombinant yeast derived HBsAg. Animals immunized with HBsAg + 1018 ISS generated anti-ssDNA antibodies 3.6-fold above baseline, but in contrast did not generate significant levels of anti-dsDNA antibodies. Standard Deviation was used to assess significant changes. Statistical analysis was done with log transformed data by analysis of variance (b) (4) using One-Way ANOVA Planned Comparison ($\alpha = 0.05$). $p < 0.05$ was considered significant

Reviewer comments and questions:

1. In this mouse study, it is not clear whether the HBsAg used for (b) (4) is the same as the one for immunization of the mice. If they are the same, the higher immune response to IgG2a in HBsAg + 1018 ISS immunized mice could be false positive. Even though subsequent studies in baboons demonstrated significant immune responses when a commercial (b) (4) test kit was used, it will still be good to know the source of the HBsAg used for (b) (4) in this mouse study so that data generated and reported in this study could be justified. Therefore, CBER sent below question to the sponsor on July 26, 2012:

On page 3, please clarify whether the HBsAg used for (b) (4) is the same as the one for immunization of the mice. If not, what is the source for HBsAg used to (b) (4) ?

The sponsor responded CBER's above inquiry on Aug 9 2012 (amend. #003). The sponsor confirmed that the (b) (4) antigen used for the mouse antibody (b) (4) and is the same as the one for immunization of the mice. Based on this piece of information, the sponsor should keep in mind that the reported antibody responses elicited by immunization with HBsAg, HBsAg & (b) (4) and HBsAg & 1018 ISS could be higher than the actual value, even though this is not a major issue at this stage of the vaccine development.

Study No. 05-446

Purpose of experiment:

(b) (4)

(b) (4)

(b) (4)

Study No. 99-0089

Purpose of Experiment:

- To determine the immune responses of baboons immunized with 1018 ISS mixed with recombinant yeast derived HBV antigen (as determined by antibody titers to HBsAg).
- To determine if baboons immunized with HBV + 1018 ISS generate antibodies to double stranded DNA (anti-dsDNA).
- To evaluate the clinical pathology of baboons as characterized by the serum chemistry and hematology analysis from a pre-immunization bleed, and from bleeds collected post-immunization with HBV + 1018 ISS at 300 µg and 3000 µg.

Summary:

- Baboons immunized twice (at 0 and 8 weeks) with recombinant yeast derived HBsAg (20 µg) mixed with 1018 ISS (3000 µg) generated 44-fold higher IgG titers than animals immunized with antigen alone, or antigen adjuvanted with (b) (4). Co-administration of HBsAg + 1018 ISS boosted the response of baboons to recombinant yeast derived HBsAg.
- Co-administration of HBsAg + 1018 ISS did not induce substantial anti-dsDNA antibodies
- Co-administration of HBsAg + 1018 ISS did not induce significant changes in the serum chemistry or hematology in most of the study baboons. For those that did show significant changes in some of the readings of serum chemistry or hematology, the sponsor stated that these changes were not due to the administration of any of the immunizations given as outlined in the study protocol, but due to blood sampling, stress response at the time of sedation, or some minor muscle trauma or traumatic tail injury.

Reviewer comments:

No deficiencies were identified with this study.

Study No. 00-104

Purpose of experiment

The objectives of this experiment were to test the antibody responses of baboons to HBV-(b) (4) formulation (yeast recombinant HBsAg formulated in PBS in the presence of 0.01% (b) (4) 80 to match the formulation to be used in the clinical trials), and to test HBV-(b) (4) formulation in combination with increasing doses 1018 ISS, in order to define the optimum dose response for immunostimulatory oligonucleotide.

Summary

Groups of 5 baboons were immunized with one of the following formulations: 20 µg HBV-(b) (4) alone, 20 µg of HBV-(b) (4) in combination with (b) (4) µg, 1000 µg or 3000 µg 1018 ISS, or 20 µg of HBV-(b) (4) + (b) (4), or Engerix-B containing 20 µg HBsAg and 0.5 mg alum, in a total volume of 1 ml. Immunizations were by the intramuscular route in the thigh. All vaccines were administered at 0 and at 2 months by intramuscular injection. Antibody responses were measured in baboons 2 weeks after each immunization. The results from this experiment indicate that after two immunizations with HBV-(b) (4) formulation in combination with (b) (4) µg, 1000 µg or 3000 µg of 1018 ISS generates an antibody response in baboons that is dose dependent. HBV-(b) (4) + 1000 µg or 3000 µg 1018 ISS is significantly more immunogenic than HBV-(b) (4) alone or the licensed vaccine Engerix-B®. After two immunizations, HBV-(b) (4) + 3000 µg 1018 ISS provided optimal antibody responses, with 100 % of the animals having protective antibody (>10 mIU/ml). This experiment confirmed the activity of HBV-(b) (4) formulation and the optimal dose range of 1018 ISS to be tested in the first clinical trial.

Reviewer comments:

No deficiencies were identified with this study.

Study No. 03-322

Purpose of experiment:

The objective of this experiment is to compare the immunogenicity of a newly sourced HBsAg antigen (*adrHBsAg*) to a previously sourced antigen (*adwHBsAg*).

Summary:

8 baboons/group were immunized twice intramuscularly (IM, quadricep) at two month intervals with: *adrHBsAg* (20 µg), *adrHBsAg* (20 µg) + 1018 ISS (3000 µg), and *adwHBsAg* (20 µg) + 1018 ISS (3000 µg). HBsAg-specific titers were determined from sera taken immediately prior to each injection and 2 weeks post each injection. *adrHBsAg* antigen in combination with 1018 ISS generated a strong immune response in baboons that was 200-fold higher than *adrHBsAg* antigen alone. The mean antibody titer generated by *adwHBsAg* +1018 ISS control vaccine group was about three times as high as that generated by *adrHBsAg* + 1018 ISS vaccine group. Although titers were higher using *adwHBsAg* + 1018 ISS the sponsor concluded that *adrHBsAg* +1018 ISS generated anti-HBsAg titers and seroprotection rates equivalent to the *adwHBsAg* + 1018 ISS control vaccine.


Reviewer comments:

Table 3 (page 6) shows that the mean antibody titer generated by *adwHBsAg* +1018 ISS control vaccine group was about three times as high as that generated by *adrHBsAg* + 1018 ISS vaccine group. The sponsor did run statistical analysis (one-way ANOVA with Fisher's LSD Multiple-Comparison) and found that the difference was not significant. Nevertheless,

the sample size may not be big enough to allow sufficient statistical power to identify the difference. Since the purpose of the experiment is to compare the immunogenicity of *adrHBsAg* to *adwHBsAg*, a larger sample size may be needed to carry the experiments to fit the purpose at that point of time. I did notice that in **Study No. 06-505** (see below), the comparison of antibody responses of baboons to adw- and adr-HBsAg in combination with 1018 ISS was carried out again and it was determined that the antibody response to adw-HBsAg was statistically significantly higher than that of adr-HBsAg at week 2 (2 weeks post-1st immunization), week 4 (4 weeks post-1st immunization) and week 6 (2 weeks post-2nd immunization). Data from Study No. 06-505 should be sufficient to supplement what is missing in the present study.

Study No. 04-416

(b) (4)



Study No. 06-505

Purpose of experiment:

The objective of this experiment is to compare the antibody responses of baboons to hepatitis B surface antigen (recombinant *Hansenula polymorpha* yeast derived) from Rhein Biotech GmbH (HBsAg (b) (4)

in combination with 1018 ISS immunostimulatory oligonucleotide.

Summary:

This study consisted of 4 groups of 10 baboons each and tested the following: 20 µg HBsAg adw^{(b) (4)}, 20 µg HBsAg adw^{(b) (4)} + 3000 µg 1018 ISS, 20 µg HBsAg adw^{(b) (4)} + 3000 µg 1018 ISS, and 20 µg HBsAg adr^{(b) (4)} + 3000 µg 1018 ISS. In previous baboon studies with HBsAg (adw^{(b) (4)} and adr^{(b) (4)}) animals were immunized by intramuscular injection (IM) in the thigh at Weeks 0 and 8 regimen. However, in the current study, baboons were immunized IM at Weeks 0 and 4, the schedule that was currently being developed for human trials with Dynavax hepatitis B vaccine. Serum HBsAg-specific antibody titers were determined from clotted blood samples taken immediately prior (Week 0), and 2 weeks post-injection (Weeks 2, and 6) and 4 weeks post-injection (Weeks 4 and 8). The results of this study demonstrated that the addition of the immunostimulatory sequence 1018 ISS significantly increases the immunogenicity of HBsAg irrespective of the antigen subtype considered. Both of the vaccines with the antigen subtype adw (HBsAg adw^{(b) (4)} + 1018 ISS and HBsAg adw^{(b) (4)} + 1018 ISS) were significantly superior to the vaccine with the antigen subtype adr (HBsAg adr^{(b) (4)} + 1018 ISS). The best overall response (highest mean antibody concentrations at every week) was with the vaccine HBsAg adw^{(b) (4)} + 1018 ISS. Thus, Dynavax plans to use HBsAg adw^{(b) (4)} for future clinical development.

Reviewer comments:

No deficiencies were identified with this study.

Study No. 99-0039

Purpose of experiment:

To compare 1018 ISS and control oligonucleotides at various doses for induction of serum cytokines following a single injection.

Summary:

In vivo cytokine response: At 2 hours post injection with 1018 ISS, increased levels of TNFα, IL-6 and IL-12(p40) were induced compared to PBS controls. IL-12(p40) increases were seen starting at the 7.8 µg dose, IL-6 increases began with the 19.5 µg dose and TNFα increases were seen starting at the 39 µg dose. At the 8 hour time point, TNFα cytokine levels had returned to baseline and the IL-6 levels had dropped 2-5 fold, but were still elevated. IL12 (p40) levels remained virtually unchanged from the 2 hour time point and reached levels several fold higher than those generated in the LPS control. No IFNγ was induced at any dose or time point. At 2 hours post injection with the (b) (4) non-ISS control oligonucleotides, there was no induction of IFNγ, TNFα or IL-6 at any dose tested. Slight induction above the PBS background of IL-12(p40) was seen at some oligonucleotide doses, but they were at least 25-fold lower than those seen in the 1018-ISS group. By 8 hours post injection, there are no elevations in cytokine levels compared to PBS controls.

Reviewer comments:

No deficiencies were identified with this study.

Study No. 04-365

Purpose of experiment:

To evaluate the serum cytokine response of rats with escalating doses of 1018 ISS.

Summary:

1018 ISS induces a dose-dependent increase of the key Th1 initiating cytokine IL-12, as was observed in mice. Rats dosed with 1500 µg 1018 ISS had the greatest serum IL-12 levels while the serum IFN γ , IL-6, and TNF α levels remained undetectable. Data from this study confirms that 1018 ISS induces the key Th1 initiating cytokine IL-12 in a dose dependant fashion in rats.

Reviewer comments:

No deficiencies were identified with this study.

Study No. hPBM-1

Purpose of experiment:

To determine if ISS has immunostimulatory activity on human PBMCs.

Summary:

Proliferation: There was marked proliferation by human PBMCs following *in vitro* treatment with ISS. This indicates that ISS has activity in human cells.

In vitro cytokine response: Secretion of significantly higher levels of IL-6 by human PBMC treated with ISS compared to non-ISS treated cells also indicates ISS has activity in human cells.

Review comments:

No deficiencies were identified with this study.

Experiment No. 182/183

Purpose of experiment:

To assay a CpG motif-containing oligodeoxyribonucleotide (1018 ISS) for IFN- γ - and IFN- α -inducing activity on human PBMCs.

Summary:

PBMCs were from eight healthy volunteers. PBMCs were resuspended in media and stimulated with 20 µg/ml 1018 ISS or 20 µg/ml (b) (4) (a non-ISS oligonucleotide) for 24 h. Supernatants were tested for IFN- γ and IFN- α levels by (b) (4). All samples were tested in duplicate (b) (4) and the concentrations were reported as the mean of the two values. Statistics were performed with (b) (4) using One-Way ANOVA with paired, nonparametric values. $p < 0.05$ is considered statistically significant. The 1018 ISS oligo significantly elevates IFN- γ and IFN- α production from human PBMCs in a CpG-specific manner.

Reviewer comments:

No deficiencies were identified with this study.

Experiment No. 194

Purpose of experiment:

To determine whether the cytokines IFN- γ and IFN- α . are up-regulated by ISS oligos at the RNA level and if so, whether that also correlates with an increase in the gene expression of certain IFN- α -inducible factors by ISS.

Summary:

PBMCs were from two healthy volunteers. PBMCs were cultured at $0.5 \times 10^6/250 \mu\text{l}$ with media alone, 10 $\mu\text{g/ml}$ 1018 ISS, or 10 $\mu\text{g/ml}$ (b) (4) non-ISS oligonucleotide. Cells were harvested at 6, 16, and 24 h, according to optimal expression of the appropriate gene and RNA was isolated using the (b) (4) and then cDNA synthesis performed using (b) (4). PCR was performed using (b) (4) techniques. (b) (4) primers used were for the human sequences of IFN- α . (6 h), IFN- γ (16 h), and three genes known to be induced in vivo by IFN- α .: 2,5 oligoadenylate synthetase, interferon-stimulated gene-54K, and guanylate-binding protein-1 (24 h). Data are expressed as the expression of each gene relative to HPRT (the (b) (4) reference gene, hypoxanthine phosphoribosyl-transferase I) in arbitrary units, and as the fold increase of expression of each stimulatory condition (1018 ISS, (b) (4) above the control condition (medium), which has been assigned a value of 1.

Results: Stimulation with 1018 ISS markedly increased the expression of IFN- γ and IFN- α . mRNA relative to (b) (4) and also substantially enhanced the levels of interferon- α -inducible genes 2,5-OAS, ISG-54K, and GBP-1. (b) (4) had very little effect on the transcription of any of these genes compared to medium stimulation alone, indicating the ISS specificity of the activity.

Reviewer comments:

No deficiencies were identified with this study.

Experiment No. 172/176/181/191

Purpose of experiment:

To assay a CpG motif-containing oligodeoxyribonucleotide (1018 ISS) for proliferation-inducing activity on human B cells.

Summary:

PBMCs were from 8 healthy volunteers. B cells were isolated by incubating PBMCs with CD19+ (b) (4) (anti-CD19 antibody conjugated to (b) (4)) and then separating B cells through (b) (4) selection. Purity of CD19+ B cells was 98-99%. B cells were cultured at $1 \times 10^5/(b) (4)$ for 72 hours with medium alone, 2 $\mu\text{g/ml}$ 1018 ISS, or 2 $\mu\text{g/ml}$ (b) (4) non-ISS oligonucleotide. After 72 hours, (b) (4) for 8 hours with (b) (4), then harvested and analyzed using standard (b) (4) techniques. Statistics were performed with (b) (4) using One-Way ANOVA with paired, nonparametric values. For the following study, $p < 0.05$ is considered statistically significant. Results: Treatment with 1018 ISS resulted in a 66-fold increase in proliferation over background counts. The 1018 ISS oligo stimulates the proliferation of human B cells 7-

fold higher than the control (b) (4) oligo. The control ODN (b) (4) also induced some proliferation, almost 10-fold above background levels, although not statistically significant ($p > 0.05$, medium vs. (b) (4)). Nevertheless, ISS-specific enhancement of proliferation was substantially greater than the level of ISS-nonspecific proliferation induced by (b) (4). Thus, it appears that although phosphorothioate ODNs can in general increase B cell proliferation somewhat, CpG motifs are required for further and substantial enhancement.

Reviewer comments:

No deficiencies were identified with this study.

Experiment No. 176/181

Purpose of experiment:

To assay a CpG motif-containing oligodeoxyribonucleotide (1018 ISS) for IL-6- and TNF- α -inducing activity on human B cells.

Summary:

PBMCs were from 4 healthy volunteers. B cells were isolated by incubating PBMC with CD19+ (b) (4) (anti-CD19 antibody conjugated to (b) (4)) and then separating B cells through (b) (4) selection. Purity of CD19+ B cells was 98-99%. B cells were cultured at 2×10^6 /ml for 48 hours with media alone, 2 μ g/ml 1018 ISS, or 2 μ g/ml (b) (4) non-ISS oligonucleotide. Supernatants were tested for IL-6 and TNF- α levels by (b) (4). All samples were tested in duplicate (b) (4) and the concentrations were reported as the mean of the two values. Statistics were performed with (b) (4) using One-Way ANOVA with paired, nonparametric values. For the following study, $p < 0.05$ is considered statistically significant. Results: Stimulation with 1018 ISS oligonucleotide resulted in the induction of IL-6 (248 pg/ml) and TNF- α (69 pg/ml) secretion by B cells. The non-CpG control oligonucleotide (b) (4) induced lower levels of these two cytokines (111 pg/ml IL-6 and 23 pg/ml TNF- α), although still higher than background. These results were similar to those observed in the B cell proliferation experiment (Experiment No. 172/176/181/191). That is, although the non-ISS ODN (b) (4) was able to induce modest secretion of IL-6 and TNF- α from human B cells ($p > 0.05$, medium vs. (b) (4)), the 1018 ISS ODN elevated those levels substantially beyond that point ($p < 0.05$, medium vs. 1018 ISS).

Reviewer comments:

No deficiencies were identified with this study.

Doc: TR-2011-01-v1: Genome Data Base Search for 1018 ISS

1. Summary

A search was undertaken to determine the extent to which 1018 ISS has identity with sequences within the known human gene and transcript databases at the NCBI (National Center for Biotechnology Information). The searches were intended to identify targets that the 1018 ISS sequence might bind to in a complementary fashion, with the assumption that binding could lead to mRNA cleavage or modulation of mRNA translation efficiency with consequent inhibition of human gene products and functions. The results indicate that there is no significant homology of the 1018 ISS sequence to targets that might affect gene

function. Therefore, the likelihood for off target effects by 1018 ISS is unlikely.

2. Materials

- BLAST nucleotide (BLASTN) search program (web-based), version 2.2.25.
- BLAST search databases:
 - Homo sapiens build 37 genome database (reference assembly GRCh37.p2); Nov 1, 2010 3:56 PM
 - Homo sapiens build 37 genome database (alternate assembly HuRef only); Nov 1, 2010 3:56 PM
 - Homo sapiens build 37 version 2 RNA database; Nov 1, 2010 3:56 PM
- Firefox web browser, version 4.0.1

3. Methods

1018 ISS is a 22 nucleotide oligomer with the sequence: 5' - TGA CTG TGA ACG TTC GAG ATG A - 3'. Using 1018 ISS as a query sequence, the NCBI BLAST database was searched using a web-based (internet web browser) interface (BLASTN version 2.2.25+). The Human Genomic Plus Transcript (Human G+T) database and the Transcript Reference Sequences (refseq_rna) database were searched. Default search parameters were used but BLAST did adjust the search for the input (query) of a short sequence. Genomic sequences with similarity to 1018 ISS were viewed using NCBI Map View to determine if the matching sequences were contained within the introns or exons of a gene, or intergenic regions. Homologous mRNA sequences were identified as matching sense or anti-sense strand of mRNA sequences.

4. Results and Discussion

A summary of the results from the BLAST searches are presented in Table 1 (Page 5-6). Primary data consisting of printouts from the web-based search can be found in Section 7 of this study report. The data indicate that there were no homologies to 1018 ISS at the level of 16 to 22 contiguous nucleotides. There were two sequences with 15 nucleotides of contiguous homology found in genomic DNA. Both of these homologous sequences were found within introns of their respective genes as determined by viewing the site of homology within the human genome using NCBI Map Viewer (see Appendix in Section 7.2). At the level of 14 contiguous nucleotides, 21 homologous sequences identified. One sequence was an mRNA that was homologous to the sense strand and would not be anticipated to have off-target effects. The other 20 homologous sequences were matches with genomic DNA. Nineteen of the 20 homologous genomic sequences were found within introns or intergenic regions, and consequently are anticipated not to have off target effects. One genomic sequence (NCBI identifier NW_001839239.1) matched the same sequence as that found in the mRNA sequence (NCBI identifier NM_0033446.1). Similarly, since the match was to the sense strand, there would be no anticipated off-target effects. Homology of less than 14 nucleotides were identified but not analyzed further. The reason for not analyzing sequences containing less than 14 nucleotides of homology is due to the lack of thermal stability with such short oligonucleotides that will prevent them from forming stable hybrids with target sequences. Overall, given the lack of identified targets that would cause 1018 ISS to interact as anti-sense oligonucleotide, the data from genome searches indicate that it is unlikely for 1018 ISS to have off-target effects.

Reviewer comments:

I have no issues with this study.