### Safety Pharmacology

PROJECT 15 is an ADC molecule and, as such, in vitro safety pharmacology assessments were limited to the effects of the metabolite, L-pAF-AGL-0185-30•2NEt3, on the hERG channel. In vivo safety pharmacology assessments (cardiovascular, central nervous and respiratory systems) of PROJECT 15 were evaluated as part of the general toxicity studies.

### 4.1.3.1 Effect of L-pAF-AGL-0185-30•2NEt3 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (Study No. 150304.BHF)

The objective of this study was to examine the in vitro effects of the metabolite L-pAF-AGL- 0185-30•2NEt3 on the hERG channel current (a surrogate for IKr, the rapidly activating delayed rectifier cardiac potassium current) at near-physiological temperature.

The metabolite, L-pAF-AGL-0185-30•2NEt3, inhibited hERG current (mean ± SEM; n = 3) by 2.2 ± 0.3% at 1 µM, and 3.4 ± 0.1% at 3 µM, versus 1.4 ± 0.7% in control. hERG inhibition at 3 µM was statistically significant (p < 0.05) when compared to vehicle control values. The half maximal inhibitory concentration (IC50) for the inhibitory effect of L-pAF- AGL-0185-30•2NEt3 on hERG potassium current was not calculated but was estimated to be greater than 3 µM (> 18000 x assuming at least a 100000 fold lower concentration for the metabolite compared to the intact ADC, in subjects at the proposed starting dose of

0.8 mg/kg).

### 4.1.3.2 A Multiple Dose Toxicity Study of PROJECT 15 Administered by Intravenous Infusion to Cynomolgus Monkeys with a 6-week Recovery Period (Study No. 20076945)

Cynomolgus monkeys were administered once weekly PROJECT 15 for 4 weeks, followed by a 6 week recovery period. No abnormal behavior and no changes in blood pressure, electrocardiography (ECG), heart rate, body temperature or respiratory rate were noted following intravenous administration of PROJECT 15 at doses up to 24 mg/kg. These observations indicate that there were no overt effects of PROJECT 15 at 24 mg/kg on the cardiovascular, central nervous or respiratory systems.

## Toxicology

Multiple studies were conducted to characterize the toxicity of the ADC, PROJECT 15 and its metabolite. The initial toxicity of the metabolite was evaluated in both rat and monkey as single-dose studies, and a study was also conducted to investigate hERG liability. A non- GLP multiple-dose toxicity study was conducted in cynomolgus monkeys to provide early information regarding toxicity and TK of PROJECT 15. The IND-enabling GLP study was conducted to define the toxicity of the ADC (PROJECT 15), unconjugated antibody (PROJECT 15) and the metabolite (pAF-AGL-0185-30). A list of the studies conducted is provided in [Table 12](#_bookmark59) and summarized below. The cynomolgus monkey was chosen as an appropriate species for toxicology studies based on comparable target sequence to human FLT3, and similar binding affinity to human FTL3. Early pharmacological studies demonstrated that

PROJECT 15 binds to human FLT3 and the antibody, PROJECT 15, cross reacts with the cynomolgus monkey ortholog with similar affinity but does not bind to the rodent ortholog.

Refer to [End-of-Text Table 3.1] and [End-of-Text Table 3.2.1].

PROJECT 15 CONFIDENTIAL

Acute Myeloid Leukemia Investigator’s Brochure

Edition 3.0

### Table 12 Toxicology Studies Conducted with PROJECT 15 or the Metabolite pAF-AGL-0185-30

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Species and Strain** | **Method of Administration** | **Duration of Dosing** | **Doses (mg/kg)** | **GLP**  **Compliance** | **Testing Facility** | **Study No.** |
| **Repeat-Dose Toxicity** |  |  |  |  |  |  |  |
| 4-Week Toxicity Study of PROJECT 15-mcMMAF, PROJECT 15, and Project 15AF-AGL-0140-19 in  Cynomolgus Monkeys with a 6-Week Recovery Period | Cynomolgus Monkeys | Intravenous infusion  – 30 min | 4 weeks | PROJECT 15: 12, 18, 24 | No | SNBL-USA | SNBL.321.02 |
| A Multiple Dose Toxicity Study of PROJECT 15 Administered by Intravenous Infusion to Cynomolgus Monkeys with a 6-Week Recovery Period | Cynomolgus Monkey | Intravenous infusion  – 30 min | 4 weeks | 12, 18, 24 | Yes | CRL-  Nevada | 20076945 |
| **Other Toxicology** |  |  |  |  |  |  |  |
| **Studies on Metabolites** |  |  |  |  |  |  |  |
| A Single Dose Study of pAF-AGL-0185-30 by Intravenous Injection in  Sprague Dawley Rats | Sprague Dawley Rats | Intravenous slow bolus | 1 week | 0.5, 1.0, 1.5 | Yes | CRL-  Nevada | 20074683 |
| A Single Dose Study of pAF-AGL-0185-30 by Intravenous Bolus Injection in Cynomolgus Monkeys | Cynomolgus Monkey | Intravenous slow bolus | 1 week | 0.083, 0.167,  0.334 | Yes | CRL-  Nevada | 20074684 |
| **Other** |  |  |  |  |  |  |  |
| Assessment of the Potential Cross Reactivity of PROJECT 15 with a Selected Panel of Human and Cynomolgus Monkey Tissues | Normal cynomolgus monkey and human tissue | Tissue titration - frozen tissue | NA | 0, 0.125,  0.625, 0.3125  µg/mL | Yes | Covance- Harrogate | 8324806 |

CRL: Charles River Laboratories; mcMMAF: maleimidocaproyl-monomethylauristatin F;NA: not applicable; pAF: para-acetyl phenylalanine; SNBL: Shin Nippon Biomedical Laboratories

Apr 2019 Astellas Page 45 of 83

### Single-dose Toxicity

No single dose studies have been conducted with the ADC or unconjugated antibody, PROJECT 15. See Section [4.3.7](#_bookmark70) below for single dose studies of the primary metabolite in rats and monkeys.

### Repeat-dose Toxicity

* + - 1. **4-Week Toxicity Study of PROJECT 15-mcMMAF, PROJECT 15, and Project 15AF-AGL- 0140-19 in Cynomolgus Monkeys with a 6-Week Recovery Period**

**(Non-GLP Study)**

* + - * 1. **Objective**

The objective of this study was to compare the toxicity of PROJECT 15 to another PROJECT 15 ADC using monomethyl auristatin F (MMAF) as the payload with a DAR of 4 in cynomolgus monkeys. The toxicity of PROJECT 15 was also compared to that of another ADC containing a different payload, AGL-0149-19, linked to PROJECT 15. The ADC was administered intravenously once weekly for 4 consecutive weeks followed by a 6-week recovery period to assess reversibility of effects. For the purposes of this document, the summary of findings below focuses on PROJECT 15 only.

### Method

The study design is summarized in [Table 13](#_bookmark63) below.

### Table 13 Experimental Design of Non-GLP Study

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | **Test Article** | **Dose Level (mg/kg)** | **Number of Animals (Male/Female)** | **Necropsy (Male/Female)** | |
| **Terminal (day 29)** | **Recovery (day 70)** |
| 1 | Control Article | 0 | 2/2 | 1/1 | 1/1 |
| 3 | PROJECT 15 | 12 | 1/1 | 1/1 | 0/0 |
| 4 | PROJECT 15 | 18 | 1/1 | 1/1 | 0/0 |
| 5 | PROJECT 15 | 24 | 2/2 | 1/1 | 1/1 |

Study assessments included: twice daily clinical observations; daily food consumption; body weight (twice in acclimation, once weekly throughout the study, and once on the day of necropsy); electrocardiography (once during acclimation and on day 23); ophthalmology (once during acclimation and on days 9 and 23); urinalysis and coagulation (once during acclimation and on days 26 and 70); hematology and serum chemistry (once during acclimation and on days 4, 11, 25, and 70). Blood was also drawn at regular intervals for TK analysis.

At necropsy gross observations were recorded, organ weights and specific tissues were collected. Histopathological analysis was conducted on sections of collected tissues stained with hematoxylin and eosin.

### Results

Intravenous administration of PROJECT 15 (12, 18, 24 mg/kg) to cynomolgus monkeys once every week for four consecutive weeks was well tolerated. No mortalities occurred and no test article-related changes were observed in urinalysis, coagulation, electrocardiography, ophthalmology, and organ weight assessments. Test article-related postural changes (hunched) were noted in PROJECT 15, 24 mg/kg group, and reductions in food consumption associated with minimal decreases in body weight were observed. Test article-related changes in hematology included decreased red cell mass (red blood cell [RBC], hemoglobin, and/or hematocrit) in one PROJECT 15- 24 mg/kg treated male on days 25 and 70, accompanied by increased red blood cell distribution width on day 70. Monocytes were mildly increased in one PROJECT 15- 24 mg/kg male on day 70. Test article related changes in serum chemistry data included minimally decreased albumin and the A:G ratio in the 24 mg/kg PROJECT 15 group on days 25 and 70. Globulin was minimally increased in one male (PROJECT 15-

18 mg/kg) on day 25, one male (PROJECT 15; - 24 mg/kg) on days 25 and 70. AST, ALT, and CK were minimally to moderately increased in multiple animals across all test article-treated groups. Increased ALT and AST may be related to hepatic changes noted on histopathology but also may be due to muscle damage (consistent with the increased CK); these increases tended to be highest in the animals treated with PROJECT 15 at 24 mg/kg.

Peak serum concentrations were generally attained instantaneously upon completion of intravenous injection for each of the three test articles. The area under the serum concentration curve (AUCτ) and Cmax for both ADC and TAb for PROJECT 15 showed dose- dependent increases, approximately proportional to dose. The exposure of PROJECT 15 determined using TAb concentrations was similar to the corresponding ADC profiles, indicating negligible deconjugation of the ADC. Serum concentrations of pAF-AGL-0185- 30 (the metabolite released from PROJECT 15) increased gradually after dosing, reached a maximum by 6 to 168 h postdose, and were > 900000-fold lower than ADC (PROJECT 15) and TAb (PROJECT 15) concentrations.

At termination, both animals at the 24 mg/kg dose exhibited mild to moderate and reversible decreased cellularity of the splenic white pulp. Following the 6-week recovery period the male but not female recovery animal had moderate multifocal mesangio-proliferative glomerulopathy, mild thickening of Bowman’s capsule, mild tubular basophilia, tubular casts and mixed inflammatory cell infiltrates. In addition, the male recovery animal at 24 mg/kg had mild multifocal centrilobular hypertrophy and sinusoidal ectasia in the liver. Based on the minimal to moderate decrease and reversible changes in hematology parameters including RBC mass, monocytes and increased ALT, AST, CK and the microscopic kidney and liver changes noted at 24 mg/kg, the no observable adverse effect level (NOAEL) for PROJECT 15 was considered to be 18 mg/kg. The results of this study provided guidance for dose levels to be used in the repeat-dose GLP monkey study described below.

### A Multiple Dose Toxicity Study of PROJECT 15 Administered by Intravenous Infusion to Cynomolgus Monkeys with a 6-Week Recovery Period (GLP Study)

* + - * 1. **Objectives**

The objectives of this study were to determine the potential toxicity and TK profiles of PROJECT 15 when administered once weekly for a total of 4 doses by a 30-min intravenous infusion to cynomolgus monkeys, and to evaluate recovery from any effects over a test article free period of 6 weeks. To aid in determination of the ADC toxicity, the unconjugated antibody (PROJECT 15) was dosed at the equivalent high dose of the ADC and the metabolite (pAF-AGL-0185-30) was administered at the molar equivalent of the high dose group.

### Method

The study design is summarized in [Table 14](#_bookmark65).

### Table 14 Experimental Design of GLP Study

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group No.** | **Test Material** | **Dose Level (mg/kg/dose)** | **Dose Volume (mL/kg)** | **Dose Concentration (mg/mL)** | **No. of Animalsa** | | | |
| **Main Study** | | **Recovery Study** | |
| **Males** | **Females** | **Males** | **Females** |
| 1 | Vehicle | 0 | 5 | 0 | 3 | 3 | 2 | 2 |
| 2 | PROJECT 15 | 12 | 5 | 2.4 | 3 | 3 | 2 | 2 |
| 3 | 18 | 5 | 3.6 | 3 | 3 | 2 | 2 |
| 4 | 24 | 5 | 4.8 | 3 | 3 | 2 | 2 |
| 5 | PROJECT 15 | 24 | 5 | 4.8 | 3 | 3 | 2 | 2 |
| 6 | pAF-AGL- 0185-30 | 0.334 | 0.334 | 1 | 3 | 3 | 2 | 2 |

pAF: para-acetyl phenylalanine

a Main study and recovery animals were necropsied on days 29 and 71, respectively.

The following parameters and end points were evaluated in this study: clinical signs and food consumption, body weights, ophthalmology, physical examinations, electrocardiography, blood pressure, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), TK and anti-therapeutic antibody analysis, gross necropsy findings, organ weights, and histopathologic examinations.

### Results

All animals survived for the duration of the study.

There were no PROJECT 15-, PROJECT 15-, or pAF-AGL-0185-30-related clinical signs or effects on body weight, food consumption, physical examination parameters, ophthalmic examination, blood pressure, and electrocardiography assessment.

PROJECT 15-related changes in hematology parameters included decreased platelets in individual animals at ≥ 12 mg/kg/dose, and decreased neutrophils and monocytes at

24 mg/kg/dose associated with decreased white blood cell counts, changes in coagulation

parameters at ≥ 12 mg/kg/dose included increased fibrinogen in individual animals, and changes in clinical chemistry parameters at 18 and/or 24 mg/kg/dose included increased AST and LDH, decreased albumin, and increased globulin (resulting in decreased A:G ratio). In general, these changes were reversible. There were no PROJECT 15- or pAF- AGL-0185-30- related changes in clinical pathology parameters.

At terminal euthanasia on day 29, gross pathology findings included an irregular surface of the liver in several animals at 18 and 24 mg/kg PROJECT 15. Increased spleen weights were present at 12, 18, and 24 mg/kg PROJECT 15. Microscopic changes included liver mild multifocal sinusoidal ectasia (dilation) at 24 mg/kg PROJECT 15 (a correlate for the irregular surface); spleen mild decreased lymphoid cellularity of the white pulp at 18 and 24 mg/kg PROJECT 15, and 0.334 mg/kg pAF-AGL-0185-30; and a dose-dependent mild to moderate lymphohistiocytic cellular infiltrate of the red pulp at 12, 18, and 24 mg/kg PROJECT 15 (a correlate for the increased spleen weights). Based upon the degree of the spleen lymphohistiocytic infiltrate and the magnitude of the corresponding spleen weight increase at terminal euthanasia, the change could be considered potentially adverse. However, evidence of reversibility was observed at recovery euthanasia.

At recovery euthanasia on day 71, recovery was evident, but not yet complete, as the only change remaining was a mild lymphohistiocytic cellular infiltrate of the red pulp in one male animal in each of the 18 and 24 mg/kg PROJECT 15, and 0.334 mg/kg pAF-AGL-0185-30 groups, but females showed complete recovery.

Cmax was reported directly from the study data and was generally attained instantaneously at the end of the intravenous injection for ADC and TAb. The pharmacokinetics of ADC and TAb followed a biphasic disposition with an initial rapid distribution phase followed by a prolonged elimination phase.

The AUC and Cmax for both ADC and TAb for PROJECT 15 showed dose-dependent increases. The increase appeared to be approximately dose proportional in the 12, 18 and 24 mg/kg dose groups. The average T½ z for ADC after the first dose was calculated as 8.58 ± 3.36, 7.75 ±

2.78 and 8.86 ± 1.85 days for the 12, 18 and 24 mg/kg dose groups, respectively. Exposure of PROJECT 15 determined using ADC and TAb concentrations indicated negligible deconjugation of the ADC over time. Serum concentration of pAF-AGL-185-30 (free drug released from PROJECT 15) increased gradually after dosing and reached a maximum by 48 – 168 h post dose for PROJECT 15 and was > 500000-fold lower than ADC and TAb (PROJECT 15, parent drug) concentrations. The T1/2z of pAF-AGL-185-30 after the last dose was calculated as 15.5 ± 4.23, 15.6 ± 5.84 and 18.8 ± 7.77 days, respectively for the 12, 18 and 24 mg/kg dose levels.

The pharmacokinetics of PROJECT 15 naked antibody followed a biphasic disposition with an initial rapid distribution phase followed by a prolonged elimination phase with a T1/2z after the first and last dose calculated as 9.70  3.72 and 12.1  6.11 days, respectively.

For animals in Dose Group 6, pAF-AGL-0185-30 was dosed at 0.334 mg/kg, which was the molar equivalent dose to the payload of a 24 mg/kg PROJECT 15 infusion. The

pAF-AGL-0185-30 dose exhibited a half-life of 0.284 ± 0.158 and 0.241 ± 0.0836 days after the first and last dose, respectively.

PROJECT 15 treatment was associated with generally minimal to mild changes in laboratory parameters including decreased platelets, white blood cell counts (specifically neutrophils and monocytes), increased fibrinogen, AST, LDH and decreased A:G ratio as a result of decreased albumin and increased globulin. In general, the clinical pathology changes were reversible. Mild and recovering microscopic findings of sinusoidal ectasia in the liver and decreased cellularity of the splenic white pulp and lymphohistiocytic infiltrate of the red pulp were not considered adverse with respect to establishing the HNSTD. The HNSTD for administration of PROJECT 15 in monkeys was 24 mg/kg, 30 times greater (HED-based) than the proposed human starting dose of 0.8 mg/kg.

### Genotoxicity

Studies evaluating the genotoxicity of PROJECT 15 have not been conducted. As noted in the ICH S9 guideline (1998), these studies are not generally conducted as ADCs are not likely to interact with DNA.

Genotoxicity of the primary metabolite of PROJECT 15, AGL-0185-30, may be evaluated later in development.

### Carcinogenicity

Studies evaluating carcinogenicity of PROJECT 15 have not been conducted.

### Reproductive and Developmental Toxicity

Studies evaluating reproductive and developmental toxicity of PROJECT 15 have not been conducted to date.

### Local Tolerance

Not applicable.

### Other Toxicity Studies

### Studies on Metabolites

The drug-linker intermediate conjugated to the mAb to produce the ADC, PROJECT 15, has not previously been characterized.

Studies conducted to characterize the metabolite of PROJECT 15 demonstrated that the drug linker (AGL-0182-30) detaches from the ADC intact along with the pAF-unit to which the drug linker was conjugated; this metabolite of PROJECT 15 is referred to as pAF-AGL-0185-30. Single dose toxicology studies in rat and monkey were conducted to better understand toxicities associated with the metabolite, these are summarized below. In addition, the toxicity associated with the deconjugated metabolite was evaluated when given as repeat doses to monkey[s (see Section 4.3.2.2](#_bookmark64) above).

### A Single Dose Study of pAF-AGL-0185-30 by Intravenous Injection in Sprague Dawley Rats

**Objectives**

The objectives of this study were to determine the potential toxicity of pAF-AGL-0185-30, when given once by intravenous slow bolus injection to Sprague Dawley rats. In addition, the TK characteristics of pAF-AGL-0185-30 were determined.

### Methods

The study design is summarized in [Table 15](#_bookmark72).

### Table 15 Experimental Design for Sprague Dawley Rat Metabolite Study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group No.** | **Test Material** | **Dose Level (mg/kg/dose)** | **Dose Volume (mL/kg)** | **Dose Concentration (mg/mL)** | **No. of Animals** | |
| **Main Study Males** | **TK Males** |
| 1 | Control | 0 | 1.5 | 0 | 5 | 3 |
| 2 | pAF-AGL-0185-30 | 0.5 | 0.5 | 1.0 | 5 | 6 |
| 3 | pAF-AGL-0185-30 | 1.0 | 1 | 1.0 | 5 | 6 |
| 4 | pAF-AGL-0185-30 | 1.5 | 1.5 | 1.0 | 5 | 6 |

pAF: para-acetyl phenylalanine; TK: toxicokinetic

The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight changes, food consumption, clinical pathology parameters (hematology, coagulation, and clinical chemistry), TK parameters, gross necropsy findings, organ weights, and histopathologic examinations.

### Results

All animals survived to scheduled euthanasia. There were no pAF-AGL-0185-30-related clinical signs or changes in body weights, food consumption, or clinical pathology.

On day 15, there were no test article-related changes in organ weights, and there were no macroscopic or microscopic changes associated with the test article.

Serum concentrations from the 0.5, 1.0, and 1.5 mg/kg dose group animals were all below the lower limit of quantification (LLOQ) of the bioanalysis method after 24 h due to rapid clearance of the drug. The area under the serum concentration curve AUClast and the first observed concentration after dose administration (Cfirst) for pAF-AGL-0185-30 showed dose- dependent increases at doses of 0.5, 1.0 and 1.5 mg/kg/dose.

In conclusion, administration of pAF-AGL-0185-30 by single intravenous (slow bolus) injection was well tolerated in Sprague Dawley rats at dose levels of ≤ 1.5 mg/kg/dose. There were no pAF-AGL-0185-30-related clinical signs or changes in body weight, body weight gain, food consumption, clinical pathology, organ weight, or macroscopic or microscopic pathology. Based on these results, the NOAEL was considered to be 1.5 mg/kg (AUClast 147 day\*ng/mL), the highest dose level evaluated in this study. This dose

corresponds to the molar equivalent of the metabolite deconjugated from a 100 mg/kg dose of ADC.

### A Single Dose Study of pAF-AGL-0185-30 by Intravenous Bolus Injection in Cynomolgus Monkeys

**Objectives**

The objectives of this study were to determine the potential toxicity of pAF-AGL-0185-30 when given as a single dose by intravenous slow bolus injection to cynomolgus monkeys, and to evaluate the potential reversibility of any findings. In addition, the TK characteristics of pAF-AGL-0185-30 were determined.

### Methods

The study design is summarized in [Table 16](#_bookmark73).

### Table 16 Experimental Design for Cynomolgus Monkey Metabolite Study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Group No.** | **Test Material** | **Dose Level (mg/kg)** | **Dose Volume (mL/kg)** | **Dose Concentration (mg/mL)** | **No. of Animals** | |
| **Males** | **Females** |
| 1 | Control Article | 0 | 0.334 | 0 | 1 | 1 |
| 2 | pAF-AGL-0185-30 | 0.083 | 0.083 | 1.0 | 1 | 1 |
| 3 | pAF-AGL-0185-30 | 0.167 | 0.167 | 1.0 | 1 | 1 |
| 4 | pAF-AGL-0185-30 | 0.334 | 0.334 | 1.0 | 1 | 1 |

pAF: para-acetyl phenylalanine

The following parameters and endpoints were evaluated in this study: clinical signs, body weights, food consumption, clinical pathology parameters (hematology, coagulation, and clinical chemistry), bioanalysis and TK parameters, gross necropsy findings, and histopathologic examinations.

### Results

There were no test article-related clinical signs or changes in body weights, food consumption, clinical pathology parameters (hematology, coagulation, and clinical chemistry), macroscopic and histopathologic examinations.

Due to an apparent short half-life of the drug, a true Cmax was not observed. Therefore, a value denoted as Cfirst was reported for the data which is the first observed concentration after administration of pAF-AGL-0185-30. The area under the serum concentration curve, AUClast and Cfirst for pAF-AGL-0185-30 generally showed dose-dependent increases at doses of 0.083, 0.167 and 0.334 mg/kg. There were no apparent TK differences between male and female monkeys following intravenous injection of pAF-AGL-0185-30 at doses of 0.167 and

0.334 mg/kg.

Administration of pAF-AGL-0185-30, when given as a single dose by intravenous (slow bolus) injection to cynomolgus monkeys was well tolerated at levels of up to 0.334 mg/kg. Based on these results, the NOAEL was considered to be 0.334 mg/kg/dose. The serum

AUClast and Cfirst associated with the NOAEL were 22.6 day\*ng/mL and 858 ng/mL, respectively. This dose corresponds to the molar equivalent of the metabolite deconjugated from a 24 mg/kg dose of ADC.

### 4.3.7.2 Other Toxicology Studies

**4.3.7.2.1 Assessment of the Potential Cross Reactivity of PROJECT 15 with a Selected Panel of Human and Cynomolgus Monkey Tissues**

**Objective**

The objective of this study was to assess, using IHC techniques, the potential cross reactivity of PROJECT 15 with histologically prepared cryo-sections from a selected panel of human and cynomolgus monkey tissues.

### Methods

A selected panel of histologically normal tissues, provided by Covance Laboratories Ltd. (Harrogate, UK), was used for the assessment of potential tissue cross-reactivity. The tissues selected for examination were as follows:

Adrenal, Urinary Bladder, Blood Cells, Bone Marrow, Breast, Brain - Cerebellum, Brain - Cortex, Colon, Duodenum, Endothelium, Eye, Fallopian Tube, Gastric Antrum, Gastric Body, Heart, Ileum, Kidney1, Liver, Lung, Lymph Node, Oesophagus, Ovary, Pancreas, Parathyroid, Parotid, Peripheral Nerve, Pituitary, Placenta, Prostate, Skin, Spinal Cord, Spleen, Striated Muscle, Testis, Thymus, Thyroid, Tonsil, Ureter, Uterus - Cervix and Uterus

- Endometrium.

1 Containing glomerulus & tubules

Three donors for each tissue were used for IHC investigation.

The assessment of tissue viability indicated that the panel of human and cynomolgus monkey tissues was viable. Based on preliminary work up, the following three concentrations of PROJECT 15 were selected for use in the tissue titration: 1.25, 0.625 and 0.3125 µg/mL.

### Results

PROJECT 15 has been found to have very weak staining in tissues that express high levels of the target (data on file). Therefore, inclusion of Control Article 1 (V62-2b36) as a positive IHC staining control as part of the control titration aided in ensuring appropriate detection of target- expressing tissues.

In the control titration phase, variable cytoplasmic-specific positive staining was observed with PROJECT 15 and V62-2b36 at all concentrations examined throughout the positive xenograft tissue (EOL-1).

In the tissue titration phase, no specific, positive staining with PROJECT 15 was observed in any of the human or cynomolgus monkey tissues examined.

In both human and cynomolgus monkey tissues variable, nonspecific staining was observed with the positive control ADC (PROJECT 15 H3-1.4.1.2-mcF), or for negative-IHC staining controls 3 (antibody TBS diluent, SG15-22 mouse IgG, anti-mouse IgG reagent) and

4 (antibody TBS diluent, anti-mouse IgG reagent) in the majority of tissues examined.

No specific positive staining was observed with PROJECT 15 in any of the human and cynomolgus monkey tissues examined.

### 4.3.7.3 Discussion and Conclusions

A series of toxicology studies were performed to characterize the safety of PROJECT 15 and the metabolite in support of initiating a phase 1 clinical study in patients with FLT3 expressing hematopoietic cancers. Monkeys were considered the relevant toxicology species based on similarity of binding affinities compared to human FLT3. PROJECT 15 did not bind to the rat ortholog.

Single- and multi-dose toxicology studies were conducted to characterize the safety of PROJECT 15 or its primary metabolite in support of the phase 1 clinical study. The definitive nonclinical toxicology studies for PROJECT 15 were conducted in the cynomolgus monkey determined to be the relevant toxicology species based on comparable target sequence to human FLT3, and similar binding affinity to human FTL3.

The acute toxicity of the primary metabolite of PROJECT 15, L-pAF-AGL-0185-30•2NEt3, was evaluated in single dose studies in rats and monkeys. A single dose of the metabolite up to

1.5 mg/kg in rats and 0.334 mg/kg in monkeys was tolerated with no adverse findings noted. These single metabolite doses are molar equivalent to the amount of metabolite contained in a 100 mg/kg dose of PROJECT 15 in rats and a 24 mg/kg dose in monkeys and represent a worst case scenario where the metabolite is released in total upon dosing.

The TK data of repeat dosing of PROJECT 15 showed that the metabolite is slowly deconjugated and progressively increases in serum. The concentrations of the metabolite achieved are over 500000 fold lower than the concentration of the ADC or TAb. Therefore, the concentrations achieved in the single dose studies are large multiples of what would be expected to be achieved in a repeat dosing setting.

The metabolite was not associated with hERG liability up to 3 µM (> 18000 x assuming at least a 100000 fold lower concentration for the metabolite compared to the intact ADC, in subjects at the proposed starting dose of 0.8 mg/kg). Following repeated dosing in monkeys, there was no evidence of quantitative or qualitative changes in the ECG attributed to PROJECT 15, PROJECT 15 or the metabolite. These findings suggest that PROJECT 15 and its metabolite are not likely to cause QTc prolongation at the anticipated clinical doses.

In the definitive GLP study, monkeys were given PROJECT 15 at doses of 12, 18 and 24 mg/kg once weekly for 4 doses followed by a 4-week recovery period. The metabolite of PROJECT 15, L-pAF-AGL-0185-30•2NEt3, was also given at the molar equivalent of the 24 mg/kg dose (0.334 mg/kg). PROJECT 15 at 24 mg/kg and its metabolite were well tolerated.

Repeated dosing with PROJECT 15 was associated with minimal and reversible changes in clinical pathology parameters including decreased platelets, decreased white cell count including neutrophils, decreased fibrinogen, slightly elevated AST, LDH and decreased A:G ratio as a result of increased albumin. The microscopic changes noted in the liver and spleen were mild and reversible. None of the changes noted were considered adverse with respect to PROJECT 15. Based on the results of this study, the HNSTD for PROJECT 15 was 24 mg/kg/dose which is 30 x higher (HED-based) than the intended starting dose for the FIH study.

## 4.4 Integrated Nonclinical Overview and Conclusion: Potential Clinical Relevance

No mortality occurred and no test article-related changes were observed in urinalysis coagulation**,** electrocardiography, ophthalmology, and organ weight assessments in cynomolgus monkeys administered PROJECT 15 weekly for 4 weeks. Spleen weights were increased with an associated mild to moderate lymphohistiocyte infiltration that was deemed to be potentially adverse and likely, reversible. All other organ weights were unchanged.

Target organs of toxicity were identified based on mild and, for the most part, reversible observations as liver, spleen, kidney and possibly the hematologic system.

**Hematological changes:** Decreased red cell mass (RBC, hemoglobin, and/or hematocrit) in one animal at the 24 mg/kg dose level. Mild increase in monocyte counts in one animal at the 24 mg/kg dose level. Hematological findings were reversible by the end of the 6-week recovery period.

**Clinical chemistry:** Minimally decreased albumin and the A:G ratio at the 24 mg/kg dose level, minimal increase in globulin levels at the 18 and 24 mg/kg dose levels.

**Skeletal muscle and liver:** AST, ALT, and CK were minimally to moderately increased in multiple animals across all test article-treated groups. Increased ALT and AST associated with histological findings of mild multifocal sinusoidal ectasia (dilation) at the highest dose tested (24 mg/kg). This finding persisted in the male monkey at the end of the recovery period. Also noted at the end of the recovery period was mild multifocal centrilobular hypertrophy. Histopathological findings were not present for skeletal muscle.

**Spleen:** At termination, mild to moderate and reversible decreased cellularity of the splenic white pulp was noted at the 24 mg/kg dose level.

**Kidney:** Following the 6-week recovery period the male but not female recovery animal for the 24 mg/kg dose group, had moderate multifocal mesangio-proliferative glomerulopathy, mild thickening of Bowman’s capsule, mild tubular basophilia, tubular casts and mixed inflammatory cell infiltrates.

Based on the minimal to moderate decrease and reversible changes in hematology parameters including RBC mass, monocytes and increased ALT, AST, CK and the microscopic kidney and liver changes noted at 24 mg/kg, the NOAEL for PROJECT 15 was considered to be

18 mg/kg.

[[Table 17](#_bookmark77)] shows the exposure margins at NOAEL and LOAEL for PROJECT 15. [[Table 18](#_bookmark78)] shows PROJECT 15 mean exposure levels. [[Table 19](#_bookmark79)] summarizes potential safety concern for PROJECT 15 for key safety targets.

### Table 17 Exposure Margins at NOAEL and LOAEL for PROJECT 15

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species/ Study Duration** | **Dose (mg/kg/day)** | **Sex (M/F)** | **Cmax (ug/mL)** | | **AUC**  **(day\*µg/mL)** | | **Cmax (last dose)** | **AUC24**  **(last dose)** |
| **First Dose** | **Last Dose** | **First Dose** | **Last Dose** | **Exposure Margin†** | |
| 20076945 | Cynomolgus Monkeys / 4 weeks / intravenous | 18 | M and F | 525 +  224 | 633 +  111 | 1800  + 956 | 2620  + 576 | 4.0 | 5.1 |
| 24 | M and F | 715 +  408 | 997 +  419 | 2220  + 1140 | 3870  + 1760 | 6.3 | 7.6 |

LOAEL: lowest observed adverse effect level; NOAEL: no observed adverse effect level

†Based on 4.8 mg/day human dose. Mean Cmax 159.1 ug/mL; Mean AUC 512.3 ug.day/mL Source: (Study 20076945, Text Table 17)

### Table 18 Mean Plasma Exposure Levels of Safety Studies

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study No.** | **Species/Study Duration/ Route** | **Dose (mg/kg/day)**  **[Human Equivalent Dose]** | **Sex** | **Steady State**  **Cmax (ug/mL)** | **Steady State AUC24**  **(ug•day/mL)** | **Based on Estimated Efficacious Human Equivalent Dose** |
| **Exposure Margin†** |
| 20076945 | Cynomolgus Monkeys / 4 weeks / intravenous | 12  [3.84] | M and F | 530 + 183 | 2130 + 610 | 4.8 |
| 18  [5.76] | M and F | 633 + 111 | 2620 + 576 | 7.2 |
| 24  [7.68] | M and F | 997 + 419 | 3870 + 1760 | 9.6 |

† Based on nonclinical pharmacology dose (10 mg/kg), (Study report RD15-005), and a calculated Human Equivalent Dose of 0.8 mg/kg for mice.

Source: (Study 20076945, Text Table 17)

### Table 19 Potential Safety Concerns of PROJECT 15

|  |  |  |
| --- | --- | --- |
| **Key Safety Targets** | **Key Observations** | **Relevance to Human Usage** |
| Liver | Minimal to moderate increase in AST and ALT. Mild multifocal sinusoidal ectasia (dilation) at the highest dose tested (24 mg/kg). This finding persisted in the male monkey at the end of the recovery period. Also noted at the end of the recovery period was mild multifocal centrilobular hypertrophy. | Possible human relevance at the human dose relative to 24 mg/kg dose observed in preclinical studies as well as preliminary findings in ongoing phase 1 study in humans. |
| Kidney | Following the 6-week recovery period the male but not female recovery animal for the 24 mg/kg dose group, had moderate multifocal mesangio- proliferative glomerulopathy, mild thickening of Bowman’s capsule, mild tubular basophilia, tubular casts and mixed inflammatory cell infiltrates. | Possible human relevance at the human dose relative to 24 mg/kg dose observed in preclinical studies. |
| Heart / Cardiovascular (incl. QT) | None. | None. |
| Hematology | Decreased red cell mass (red blood cells, hemoglobin, and/or hematocrit) in one animal at the 24 mg/kg dose level. Mild increase in monocyte counts in one animal at the 24 mg/kg dose level.  Hematological findings were reversible by the end of the 6-week recovery period. | Possible neutropenia, anemia, thrombocytopenia confounded by disease based on preclinical observations as well as preliminary findings in ongoing phase 1 study. |
| Genotoxicity | Not performed. | None. |
| Reproductive toxicity | Not performed. | None. |
| Carcinogenicity | Not performed. | None. |
| Ocular toxicity | No ocular toxicity observed in animal species. | Possible corneal lesions based on preliminary findings in ongoing phase 1 study. |
| Hypersensitivity reaction/ Infusion- related toxicity | Not performed. | Possible ADC risk or development of anti-ADC antibodies; also observed in ongoing phase 1 study. |
| Peripheral sensory neuropathy | Not performed. | Possible clinical sequelae based on mechanism of action of the proprietary cytotoxic moiety. |
| *Other relevant toxicity-related information* | None. | Not applicable. |
| Indications for clinically relevant drug interactions | No drug-drug interaction studies have been performed to date. | None. |

ADC: antibody-drug conjugate