* 1. **Safety Pharmacology**

*In Vitro Study: Effect of MMAE on* human ether-a-go-go-related gene *(hERG) Current*

The effect of MMAE on hERG K+ channels, heterologously expressed in Human Embryonic Kidney (HEK) 293 cells, was evaluated using the conventional whole cell voltage clamp technique [Study 129-09-001]. The effects on hERG K+ currents were examined by measuring peak hERG tail current before and during test and control article exposure at 35±1ºC. MMAE effects at 10 and 100 μM were compared to the negative control (extracellular saline). Cisapride hydrate (25 nM) was used as a positive control. MMAE at 100 μM produced a fractional block of peak hERG tail current of 0.237±0.056 that was significantly different than the effect of the negative control (mean fractional rundown of 0.063±0.023, mean±SEM), whereas MMAE at 10 μM produced a fractional block of 0.103±0.030 that was not significantly different from the effect of the negative control. Even at the 100 μM high dose of MMAE the effect on the hERG K+ channel was insufficient to calculate an IC50, but is estimated to be greater than 100 μM.

# Toxicology

## Single-dose Toxicity

As part of the exploratory dose-range finding study in monkeys (Study No. 20053122), monkeys were given a single dose of PROJECT 16-1. The results of that study are described in Sec[tion 4.1.1.3.](#_bookmark60)

## Repeat-dose Toxicity

One GLP study in rats (20043365) and two repeat-dose studies in cynomolgus monkeys were conducted – one exploratory (20053122) and one pivotal GLP study (20043367) to characterize the safety of PROJECT 16-1 in support of initiating a Phase 1 clinical trial in patients with hematopoietic cancers associated with CD37 expression. Cynomolgus monkeys were considered the relevant toxicology species based on similarity of a variety of immunohistochemistry and flow cytometry-based binding characteristics to human CD37.

PROJECT 16-1 did not bind to the rat ortholog, however, a study was conducted with rats to characterize target independent toxicity attributed to PROJECT 16-1.

A list of toxicology studies conducted is provided [in Table 11.](#_bookmark77)

## Table 11. Toxicology Studies Conducted with PROJECT 16-1 and/or MMAE

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Study** | **Species and Strain** | **Method of Administration** | **Duration of Dosing** | **Doses a** | **GLP** | **Testing Facility** | **Study No.** |
| **Repeat-Dose Toxicity** |  |  |  |  |  |  |  |
| A Multiple Dose Toxicity Study of PROJECT 16-1 and PROJECT 16-2 Administered by IV Injection to Sprague Dawley Rats with  a 6-Week Recovery Period | SD Rat | IV bolus | 4 weeks | 0, 1, 3 and  10 mg/kg | Yes | CRL-  Nevada | 20043365 |
| An Exploratory Toxicity Study of PROJECT 16-1 Administered by IV Infusion to Cynomolgus Monkeys | Cynomolgus Monkeys | IV infusion- 30 minute | 8 weeks | 0.4, 0.8, 2.4  and 4.8 mg/kg | No | CRL-  Nevada | 20035122 |
| A Multiple Dose Toxicity Study of PROJECT 16-1 and PROJECT 16-2 Administered by IV Infusion to Cynomolgus Monkeys with  a 6-Week Recovery Period | Cynomolgus Monkey | IV infusion- 30 minute | 4 weeks | 0, 0.25,  0.5b 1/0.75  and 3 mg/kg | Yes | CRL-  Nevada | 20043367 |
| **Genotoxicity** |  |  |  |  |  |  |  |
| Reverse mutation assay | *S.*  *typhim urium* TA98, TA100  , TA1535, TA1537  *E. coli*  WP2*uvrA* | In vitro | - | -S9: 0.25 to  5000  μg/plate  +S9: 0.25 to  5000  μg/plate | Yes | BioReliance | AA66EH.5 03.BTL |
| Forward mutation assay | Mouse lymphoma L5178Y TK+/-  cell line | In vitro | - | -S9, 4h;  0.005 to 50 ng/mL  +S9, 4h; 0.05  to 100 ng/mL  -S9, 24h;  0.05 to 6.0 ng/mL | Yes | Covance Laboratories, Inc. | 8204-155 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| In vivo micronucleus test | Male SD rats | IV bolus | Single dose | 0.01, 0.1 or  0.2 mg/kg | Yes | Covance Laboratories, Inc. | 8204-151 |
| **Reproductive and Developmental Toxicity** |  |  |  |  |  |  |  |
| Embryo-fetal Development | Female SD rats | IV bolus | 1 week  (GD 6 and  13) | 0.2 mg/kg | No | Covance  Laboratories, Inc. | 8204-397 |
| **Other** |  |  |  |  |  |  |  |
| Assessment of the Potential Cross Reactivity of PROJECT 16-1 with a Selected Panel of Human and Cynomolgus Monkey  Tissues | NA | Tissue titration- frozen tissue | NA | 0, 0.16, 0.31  and 0.63 ug/mL | Yes | Covance- Harrogate | 8287084 |

CRL: Charles River Laboratories; GD: gestational day; GLP: Good Laboratory Practice; NA: not applicable; SD: Sprague Dawley

a Underline doses represent NOAEL dose

b Dose was considered to be the HNSTD under the conditions of this study

## A Multiple Dose Toxicity Study of PROJECT 16-1 and PROJECT 16-2 In Sprague Dawley Rats

A Multiple Dose Toxicity Study of PROJECT 16-1 and PROJECT 16-2 Administered by IV Injection to Sprague Dawley Rats with a 6-Week Recovery Period (Charles River Study No. 20043365).

## Objective

The objectives of this GLP study were to determine the potential toxicity and toxicokinetic (TK) profiles of PROJECT 16-1 when administered once every week for a total of 4 doses by IV bolus injection to Sprague Dawley rats, and to evaluate recovery from any effects over a dose-free period of 6 weeks. Since PROJECT 16-1 does not cross react with rat, the findings in this study address potential target independent toxicities of PROJECT 16-1.

To aid in determination of the ADC toxicity, the unconjugated antibody (PROJECT 16-2) was dosed at the equivalent of the high dose of the ADC, PROJECT 16-1.

## Methods

The study design is summarize[d in Table 12](#_bookmark78) below.

## Table 12. Experimental Design for Study 20043365

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group No.** | **Test Material** | **Dose Level (mg/kg/wk)** | **Dose Volume (mL/kg)** | **Dose Conc. (mg/mL)** | **No. of Animals** | | | |
| **Main Study** | | **Recovery Study** | |
| **Males** | **Females** | **Males** | **Females** |
| 1 | Vehicle | 0 | 5 | 0 | 10 | 10 | 5 | 5 |
| 2 | PROJECT 16-1 | 1 | 5 | 0.2 | 10 | 10 | 5 | 5 |
| 3 | PROJECT 16-1 | 3 | 5 | 0.6 | 10 | 10 | 5 | 5 |
| 4 | PROJECT 16-1 | 10 | 5 | 2 | 10 | 10 | 5 | 5 |
| 5 | PROJECT 16-2 | 10 | 5 | 2 | 10 | 10 | 5 | 5 |
| **Toxicokinetics** | | | | | | | | |
| 6 | Vehicle | 0 | 5 | 0 | 9 | 9 |  | |
| 7 | PROJECT 16-1 | 1 | 5 | 0.2 | 9 | 9 |
| 8 | PROJECT 16-1 | 3 | 5 | 0.6 | 9 | 9 |
| 9 | PROJECT 16-1 | 10 | 5 | 2 | 9 | 9 |
| 10 | PROJECT 16-2 | 10 | 5 | 2 | 9 | 9 |

PROJECT 16-1 – ADC; PROJECT 16-2 – unconjugated antibody

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, ophthalmology, clinical pathology parameters (hematology and clinical chemistry), TK parameters, ADA analysis, gross necropsy findings, organ weights, and histopathology evaluation.

## Results

PROJECT 16-1 at 1 and 3 mg/kg/week and PROJECT 16-2 at 10 mg/kg/week were generally well tolerated. There were 9 animals (4 controls [Group 6-TK], and 5 PROJECT 16-1 animals at 10 mg/kg/week [Groups 4/9]) that were found dead or euthanized prior to scheduled

necropsy. Two of the early mortalities at 10 mg/kg/week were considered related to PROJECT 16-1

administration, and the remaining mortalities were considered procedure related. One of the early mortality animals was noted with generalized pale appearance and/or decreased activity starting on day 21, and was noted with 68 g body weight loss on day 28 (compared to day 21). On day 29, this animal had marked changes in hematology parameters that included decreases in indicators of erythrocyte mass, reticulocytes, erythrocytes indices, leukocytes, and platelets. There were mild changes in clinical chemistry parameters including increases in alanine aminotansferase (ALT), aspartate aminotransferase (AST), and moderate increases in blood urea nitrogen (BUN) and total bilirubin on day 29. In general, these changes correlated with histopathological observation of single cell necrosis of hepatocytes and decreased erythroid and myeloid cellularity of the bone marrow. The other early death occurred on day 30 in a TK animal from the high dose group (10 mg/kg). No clinical pathology data were available on this animal; however, it was noted to have lost 58 g of body weight.

PROJECT 16-1-related clinical signs in animals surviving to scheduled necropsy at the 10 mg/kg/week dose level included pale skin, thin fur cover and skin lesions. Pale skin was first noted on day 21 and the last observed occurrence was on day 42. PROJECT 16-1-related decreases in body weight were observed in 10 mg/kg/week males starting from week 4 and continuing through week 7. The average food consumption was minimally lower in males at the 10 mg/kg/week dose level between weeks 3-4. PROJECT 16-1-related ocular findings included pale retina/choroid in a majority of the 10 mg/kg/week animals at week 4.

Hematology changes at 10 mg/kg/week included decreases in indicators of erythrocyte mass, reticulocytes, erythrocytes indices, leukocytes, and platelets. In general, these changes correlated with histopathological observation of minimal to marked decreased erythroid and myeloid cellularity of the bone marrow on day 29. At 3 mg/kg/week there were minimal, non-adverse PROJECT 16-1-related decreases in erythrocyte mass. There were no test article- related changes in hematology parameters at 1 mg/kg/week.

There were mild to moderate, nonadverse PROJECT 16-1-related changes in clinical chemistry parameters at 10 mg/kg/week including increases in ALT, AST, Alkaline phosphatase (ALP), and Gamma-Glutamyltransferase (GGT) on day 29 and in some animals following the 6- week recovery. BUN was also minimally increased on day 29. At 3 mg/kg/week, ALT and AST were increased during the 6-week recovery period in 2 females. There were no

PROJECT 16-1-related changes in clinical chemistry parameters at 1 mg/kg/week.

Maximum serum PROJECT 16-1 concentration was generally attained instantaneously at the end of the IV injection and showed a bi-exponential decline thereafter. Serum PROJECT 16-1 concentrations were generally higher than the ADC concentrations. Dose linear toxicokinetics (TK) was observed at doses of 1, 3 and 10 mg/kg/week. The Cmax values for both ADC and TAb after the first and last dose suggested that there is accumulation (1.5 – 2 fold) of the test article when administered weekly and this is attributed to a long half-life (T½) of the drug (T½ for ADC after the last dose calculated as 9.35, 9.35 and 8.98 days for 1, 3 and 10 mg/kg dose groups, respectively and for TAb calculated as 12.7, 12.8 and 12.1 days for 1, 3 and 10 mg/kg dose groups, respectively). Maximum MMAE metabolite

concentrations (Cmax) were attained at 24 hours post-PROJECT 16-1 dose injection. The elimination half-life (T½ z) after the last dose was calculated as 2.96, 6.55 and 7.25 days at 1, 3 and 10 mg/kg dose levels, respectively. Blood samples for monitoring immunogenicity were collected from Groups 1 to 5 and the overall incidences of seroconversion were 0%, 0%, 0%,

0%, and 3.33% for Dose Groups 1, 2, 3, 4 and 5 respectively.

At the end of the dosing phase (day 29), PROJECT 16-1-related organ weight changes were observed at 3 and 10 mg/kg/week. At the 3 and 10 mg/kg/week dose levels, thymus and testis weights (absolute and relative) were decreased, while at 10 mg/kg/week organ weight changes (absolute and relative) consisted of increased lung weights and decreased epididymis and prostate gland weights. Macroscopic findings present at 3 and 10 mg/kg/week at the end of the dosing phase (day 29) included small testes and reduced size of the epididymides and seminal vesicles. At 10 mg/kg, several l tissues (bone marrow, brain, pituitary gland, prostate gland, thyroid gland, kidneys, liver, lung, pancreas, and uterus) were noted to have pale discoloration which correlated with decreased Red blood cell (RBC) clinical pathology indices.

PROJECT 16-1-related microscopic findings were present in males at all dose levels and in males and females at 3 and 10 mg/kg/week. At 1, 3 and 10 mg/kg/week, minimal to severe degeneration of the seminiferous tubules were noted in the testes. At 3 and 10 mg/kg/week, test article-related findings included: mild to marked cellular debris within the epididymides; minimal to moderate single cell necrosis of centrilobular hepatocytes of the liver; minimal to moderate type 2 pneumocyte hyperplasia and increased numbers of alveolar macrophages within the lung; and minimal to severe hypospermatogenesis within the testes. At

10 mg/kg/week, PROJECT 16-1-related findings were: minimal to marked decreased erythroid and myeloid cellularity of the bone marrow; minimal to moderate decreased lymphocyte cellularity within the lymph nodes and Gut associated lymphoid tissue (GALT); marked thymic lymphoid depletion; minimal to mild decreased cellularity within the red pulp of the spleen; and marked atrophy of the seminiferous tubules of the testes.

At the end of the recovery period (day 64), epididymis and testes weights were decreased in males which was consistent with macroscopic evidence of small, soft testes at 1 and 3 mg/kg, and reduced size of the epididymides at 3 mg/kg/week. PROJECT 16-1-related microscopic findings at the end of the recovery period (day 64) included: minimal to moderate cellular debris within the epididymides, minimal to severe degeneration of the seminiferous tubules of the testes, and minimal to severe hypospermatogenesis at all doses; marked atrophy of the seminiferous tubules at 3 and 10 mg/kg/week; minimal to mild alveolar macrophage aggregation within the lung of females at 10 mg/kg/week; and minimal single cell necrosis of centrilobular hepatocytes in the liver of 1 male at 10 mg/kg/week.

## Conclusion

Based on the bone marrow hypocellularity and hematology changes at 10 mg/kg/week, the NOAEL for females is considered to be 3 mg/kg/week. Although in males, a NOAEL could not be strictly determined due to histopathology findings of the testis and epididymides noted at all doses at the terminal and recovery necropsies, these findings are not unexpected given

the mechanism of action of MMAE which is a microtubule disrupting agent. The bone marrow and testis lesions are consistent with the cytotoxicity associated with the free drug (MMAE) that typically targets rapidly dividing cells and has been previously observed with other MMAE-related antibody conjugates.

## An Exploratory Toxicity Study of PROJECT 16-1 in Cynomolgus Monkeys

An Exploratory Toxicity Study of PROJECT 16-1 Administered by IV Infusion to Cynomolgus Monkeys (Charles River Study No. 20035122)

## Objective

The objectives of this exploratory (non-GLP) study were to evaluate the toxicity, TK profile, and pharmacodynamics of PROJECT 16-1 when administered via IV injection to cynomolgus monkeys.

## Methods

The study was conducted in 2 parts. Phase I was designed to determine the single dose PK and pharmacodynamics of PROJECT 16-1 and to evaluate single and multiple dose toxicity.

Additional animals were added to the study for Phase II to evaluate lower doses and dose regimens based on findings from Phase I. The study design is summarize[d in Table 13](#_bookmark79) below.

## Table 13. Experimental Design of Study 20035122

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | **No. of Males (Main Study/ Recovery)** | **Test Article** | **Dose (mg/kg; actual dose)** | **Dose Volume (mL/kg)** | **Conc. (mg/mL)** | **Route** | **Necropsy (No. of Males)a** |
| **Phase I** | | | | | | | |
| 1 | 3/0 | PROJECT 16-1 | 1d (0.8) | 5 | 0.2 | IV | 3 a |
| 2 | 3/0 | PROJECT 16-1 | 3d (2.4) | 5 | 0.6 | IV | 3 a |
| 3 | 3/0 | PROJECT 16-1 | 6d (4.8) | 5 | 1.2 | IV | 3 a |
| 4 e | 2/2 | PROJECT 16-1 | 3d (2.4) | 5 | 0.6 | IV | 4 |
| 5 e | 2/2 | PROJECT 16-1 | 6d (4.8) | 5 | 1.2 | IV | 4 |
| **Phase II** | | | | | | | |
| 6 b | 3/0 | PROJECT 16-1 | 0.4 | 5 | 0.08 | IV | 3b |
| 7 c | 3/0 | PROJECT 16-1 | 0.8 | 5 | 0.16 | IV | 3c |
| 8 c | 3/0 | PROJECT 16-1 | 2.4 | 5 | 0.48 | IV | 3c |

No. = number, Conc. = concentration

a Group 1, 2 and 3 animals were continued on study and assigned to Phase II as Groups 6, 7, and 8, respectively after a 51 day washout period.

b Group 6 animals were necropsied on Day 29 (1 week after last dose).

c Group 7 and 8 animals were necropsied on Day 50 (1 week after last dose).

d Nominal dose level and dose concentrations. Per Certificate of Testing provided by the Sponsor, the actual dose levels were 0.8, 2.4, 4.8, 2.4, and 4.8 mg/kg for Groups 1, 2, 3, 4, and 5 respectively.

e All Group 4 and 5 animals were euthanized early.

In Phase I, animals in Group 1-3 received a single dose of PROJECT 16-1 and were continued on study until day 51 and reassigned to Phase II. Group 4 and 5 animals were intended to receive

weekly doses of PROJECT 16-1 for a total of 4 doses; however, due to toxicity, they only received 2 doses and were euthanized prior to scheduled necropsy.

In Phase II, Group 6 animals received weekly doses of PROJECT 16-1 for a total of 4 doses on days 1, 8, 15, and 22. Group 7 and 8 animals received a total of 4 doses every other week on days

1, 15, 29, and 43.

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), TK parameters, flow cytometry (immune cell subsets) and receptor occupancy parameters, gross necropsy findings, organ weights, and histopathologic examinations.

## Results

**Phase I of Study in Cynomolgus Monkeys**

There were no test article related changes in body weight or food consumption following a single dose of PROJECT 16-1 at 0.8, 2.4 or 4.8 mg/kg. PROJECT 16-1-related clinical signs at 4.8 mg/kg included skin lesions with scabs. Red cell mass was minimally decreased at all doses at day

22. PROJECT 16-1-related, dose-independent, transient, and recoverable decreases were present for all lymphocyte subsets at the 6 hour post-end of infusion (EOI) time point and for monocytes at the 24 hour post-EOI time point in Groups 1-3. The greatest decreases, listed in order of magnitude, were present for monocytes, B-lymphocytes, natural-killer (NK) cells, and T- lymphocytes.

Maximum serum concentrations (Cmax) for ADC and TAb upon administration of PROJECT 16-1 were attained between 5 minutes and one hour post end of 30- minute IV infusion. The TAb concentrations were generally higher in comparison to its corresponding ADC profiles. The area under the serum concentration curve [AUC(0-7days)] and Cmax for both ADC and TAb showed dose-dependent increases. The increase appeared to be approximately dose proportional at dose groups administered (0.8, 2.4 and 4.8 mg/kg). These early and limited data suggest dose linear TK; however, a definitive dose linearity relationship could not be concluded due to the limited amount of data obtained in this exploratory study. The elimination half-life (T½ z) for PROJECT 16-1 ADC and TAb was short and ranged from 1.37 –

2.83 days and 1.97 - 4.78 days across groups 1-5, respectively.

Weekly doses of PROJECT 16-1 via IV administration at 2.4 and 4.8 mg/kg on days 1 and 8 resulted in mortality and morbidity. One 4.8 mg/kg/dose animal was found dead on day 10; the remaining animals were euthanized early on days 11 and 13 due to poor health condition.

PROJECT 16-1-related clinical signs included skin lesions with scabs, hunched posture, decreased activities, starting on day 8. Changes in clinical pathology parameters included markedly decreased red cell mass, decreased reticulocyte, markedly decreased leukocytes, moderately decreased albumin and increased globulin resulting in a decreased albumin:globulin ratio, moderately increased fibrinogen in all group 4 (2.4 mg/kg) and group 5 (4.8 mg/kg) animals, and minimally increased ALT, AST, ALP, creatine kinase (CK), minimally prolonged prothrombin time and activated partial thromboplastin time in individual animals. Flow

cytometry data indicated marked reductions in all lymphocyte subsets and monocyte populations.

Histopathology findings included moderate to marked bone marrow hypocellularity (erythroid and myeloid), marked systemic lymphoid depletion characterized by a marked decrease in the number of lymphocytes in the thymus, spleen, mandibular lymph node, mesenteric lymphoid, and GALT (immunosuppression) and severe opportunistic bacterial infections. Bone marrow hypocellularity (erythroid and myeloid) was similar in incidence; however, slightly decreased in severity in Group 4 animals (moderate to marked) as compared to Group 5 animals (marked). All other macroscopic and microscopic findings in the early death animal were considered to be related to debilitation, inanition, bacterial infection, bacterial septicemia and/or circulatory failure/hypoxia (shock).

## Phase II of Study in Cynomolgus Monkeys

Following a 51-day washout period of the single dose animals from Phase I, additional dosing was conducted to further define dose and regimen. IV administration of PROJECT 16-1 at

0.4 mg/kg/weekly for a total of 4 doses or once every other week at 0.8 mg/kg/dose and

2.4 mg/kg/dose for a total of 4 doses was generally well-tolerated in cynomolgus monkeys.

There were no PROJECT 16-1-related changes in body weight or food consumption. While no PROJECT 16-1-related clinical signs were present at 0.4 or 0.8 mg/kg, two group 8 (2.4 mg/kg) animals had skin lesions on day 9 (similar to Phase I animals). There were no PROJECT 16-1- related changes in clinical chemistry, coagulation, or urinalysis parameters. In animals treated with 0.8 mg/kg once every 2 weeks, changes in hematology parameters included minimal decreases in leukocyte counts on days 7 and 14. PROJECT 16-1-related changes in hematology parameters in the group 8 animals (2.4 mg/kg) included moderately decreased red cell mass, markedly decreased leukocytes at day 7 and 49.

PROJECT 16-1-related, dose-dependent, and variable decreases were present for T- and B- lymphocyte subsets for 0.8 mg/kg and 2.4 mg/kg groups at the 6 hour post-EOI time point and for the 2.4 mg/kg group at the 24 hour post-EOI time point. T- and B-lymphocyte values for Group 7 (0.8 mg/kg) generally recovered by the day 50 time point; however, group 8 (2.4 mg/kg) values were reduced at this time point. All dose levels demonstrated minor reduction in NK-cells at the day 1- 6 hour time point. For the 0.4 mg/kg group NK cells returned to baseline by day 15 while values for the 0.8 mg/kg did not recover until day 50 and the NK- cells for 2.4 mg/kg remained decreased.

The serum concentration data for PROJECT 16-1 ADC and TAb from Group 6 was below the lower limit of quantification (LLOQ). In addition serum concentration data for PROJECT 16-1 ADC and TAb from group 7 was below the LLOQ beyond day 1 (24 h) – day 1 (96 h). However serum concentration data for PROJECT 16-1 ADC and TAb from group 8 was above the LLOQ for the entire profile. These observations could be attributed to the incidence of immunogenicity in animals from groups 6 and 7. Since immunogenicity was not tested in this study, a definitive relationship between immunogenicity and serum concentrations could not be concluded. In group 8, maximum serum concentrations for ADC and TAb upon administration of PROJECT 16-1

were attained between 5 minutes and one hour post end-of-30- minute IV infusion. The TAb concentrations were generally higher in comparison to its corresponding ADC profiles for both antibodies. The elimination half-life (T½ z) for PROJECT 16-1 ADC and TAb ranged from

1.58 – 1.98 days and 1.65 – 2.13 days for group 8. The Cmax values for both ADC and TAb after the first and last dose suggested that there is no accumulation of the test article when administered every two weeks and this is attributed to a short half-life of the drug.

PROJECT 16-1-related organ weight changes were identified in the spleen and thymus at

2.4 mg/kg. Mean spleen weight (absolute, percent of body and brain weights) as well as mean thymus weight (absolute, percent of body and brain weights) was slightly decreased in Group 8 animals as compared to the other dose groups. The decrease in spleen weight correlated with a marked decrease in number/size of lymphoid follicles/germinal centers in all animals. There was no consistent microscopic correlate for the decrease in mean thymus weight; however, minimal lymphoid necrosis was identified in 1 animal.

PROJECT 16-1-related microscopic findings identified in the lymphoid tissues and bone marrow were similar yet often less severe than those described in the early death animals from Phase

I. A mild or marked decrease in the number and size of lymphoid germinal centers (germinal center depletion) was identified in the spleen at ≥ 0.8 mg/kg (Groups 7 and 8); and in GALT, mandibular lymph node and mesenteric lymph node at 2.4 mg/kg (Group 8). In the spleen, the germinal center depletion increased in incidence and severity in a dose dependent fashion. In the thymus, minimal lymphoid necrosis was identified in 1 of 3 animals in the 2.4 mg/kg dose group. In the bone marrow, moderate or marked hypocellularity (myeloid and erythroid) and a minimal or mild increase in medullary macrophages (containing phagocytized cellular debris) were identified in all 2.4 mg/kg dose group animals. Potential PROJECT 16-1-related macroscopic findings were identified in the skin at 2.4 mg/kg. The gross observation of scales or scabs on the left forearm in two 2.4 mg/kg dose group correlated microscopically with focal ulcerative or non-ulcerative dermatitis characterized by focally extensive acanthosis (epidermal hyperplasia), hyperkeratosis, crust formation (with or without intralesional bacteria and epidermal ulceration), mixed or mononuclear perivascular inflammation and fibroplasias/fibrosis in the underlying dermis. In both animals, hair follicles were affected and rare individual apoptotic keratinocytes were present in epidermis in 1 of the 2 animals. The subcutaneous facial nodule noted in one 2.4 mg/kg dose group animal was characterized by focal epidermal necrosis overlying an area of moderate focal dermal necrosis with intralesional bacteria as well as deposition of abundant basophilic material surrounded by mixed inflammation and fibroplasias/fibrosis. Additional findings of uncertain relationship to PROJECT 16-1 administration were identified in the kidney (arteritis and glomerulonephritis) in all PROJECT 16-1 dose groups. These are relatively common background findings in cynomolgus monkeys; however, the high incidence in this study makes the relationship to test article uncertain.

## Conclusions

The results of Phase I indicated that weekly administration at ≥ 2.4 mg/kg was not tolerated as a result of marked immunosuppression and development of bacterial opportunistic

infection. In addition the skin lesions are likely secondary to immunosuppression as the target (CD37 for PROJECT 16-1) is not expressed in the skin. These results suggest that further studies should allow for recovery of lymphocyte subsets prior to further dose administration to avoid the possibility of developing opportunistic infections. Therefore for Phase II, a once every 2 week dose interval was chosen based on the data from the single dose arm of Phase I which demonstrated that the immune cells tended to recover between days 11 and 14 after dosing.

In Phase II, weekly doses of 0.4 mg/kg or once every other week doses at 0.8 and 2.4 mg/kg were generally well tolerated. Hematologic changes similar to those observed in Phase I were noted but were less severe. Treatment-related, dose-dependent and variable decreases were present at the 0.8 and 2.4 mg/kg dose levels for T and B lymphocytes which generally recovered by day 50. In contrast NK cells remained decreased at the end of the treatment period. Microscopic findings in the lymphoid tissue and bone marrow were similar to Phase I animals but were less severe. Skin lesions were noted at the 2.4 mg/kg dose level but were likely associated with bacterial infection secondary to immune suppression and not a direct effect of the test article. The results from Phase II must take into consideration that animals had decreasing levels of exposure over the study period. Although immunogenicity was not assessed in this study, it is likely that these animals developed antidrug antibodies. Therefore, the results may be understated given the variable exposure. In general, this arm of the study suggested that for the best tolerability dosing in further studies should be based on allowing immune cells to recover between doses.

## A Multiple Dose Toxicity Study Of PROJECT 16-1 And PROJECT 16-2 Administered By IV Infusion To Cynomolgus Monkeys With A 6-Week Recovery Period (Charles River Study No. 20043367)

* + - * 1. **Objective**

The objectives of this GLP study were to determine the potential toxicity and TK profiles of PROJECT 16-1 when administered once every 2 weeks for a total of 4 doses (on Days 1, 15, 29, and 43) by a 30-minute IV infusion to cynomolgus monkeys, and to evaluate recovery from any effects over a dose-free period of 6 weeks. To aid in determination of the ADC toxicity, the antibody (PROJECT 16-2) was dosed at the equivalent to the high dose of the ADC and the drug payload (the small molecule attached to the antibody, MMAE) was administered at the molar equivalent of the high dose ADC group.

## Methods

The study design is summarize[d in Table 14.](#_bookmark80)

## Table 14. Experimental Design for Study 20043367

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group No.** | **Test Material** | **Dose Level (mg/kg/dose)** | **Dose Volume (mL/kg)** | **Dose Conc. (mg/mL)** | **No. of Animals** | | | |
| **Main Study** | | **Recovery Study** | |
| **Males** | **Females** | **Males** | **Females** |
| 1 | Vehicle | 0 | 5 | 0 | 3 | 3 | 2 | 2 |
| 2 | PROJECT 16-1 | 0.5 | 5 | 0.1 | 3 | 3 | 2 | 2 |
| 3 | PROJECT 16-1 | 1/0.75a | 5 | 0.2/0.15a | 3 | 3 | 2 | 2 |
| 4b | PROJECT 16-1 | 3 | 5 | 0.6 | 3 | 3 | 2 | 2 |
| 5 | PROJECT 16-2 | 3 | 5 | 0.6 | 3 | 3 | 2 | 2 |
| 6 | MMAE | 0.05 | 1 | 0.05 | 3 | 3 | 2 | 2 |
| 7 | PROJECT 16-1 | 0.25 | 2.5 | 0.1 | 3 | 3 | 2 | 2 |

PROJECT 16-1 = ADC; PROJECT 16-2 = unconjugated antibody; MMAE = small molecule

a received 1 dose at 1 mg/kg, and the dose level was lowered to 0.75 mg/kg starting from day 15

b due to mortality and morbidity, group 4 animals received a single dose on day 1

The following parameters and end points were evaluated in this study: clinical signs, body weights, food consumption, ophthalmology, electrocardiogram (ECG), blood pressure, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), flow cytometry and receptor occupancy parameters, TK, ADA formation, gross necropsy findings, organ weights, and histopathologic examinations.

## Results

PROJECT 16-1 administration at 1 and 3 mg/kg resulted in mortality/morbidity. PROJECT 16-1 at 0.25 and 0.5 mg/kg/dose, the 0.75 mg/kg/dose (lowered from 1.0 mg/kg) from day 15, PROJECT 16-2 at 3 mg/kg/dose and MMAE at 0.05 mg/kg/dose was generally well-tolerated.

Eight PROJECT 16-1-dosed animals (1 animal at 1 mg/kg and 7 animals at 3 mg/kg/dose) were euthanized or died prior to their scheduled euthanasia. Of these, 2 animals (1 each at 1 and 3 mg/kg/dose) were found dead on day 10 and 6 animals (3 mg/kg/dose) underwent unscheduled euthanasia due to deteriorating health on days 11-15. In general, all early death animals had similar clinical signs on the days preceding the unscheduled euthanasia. These clinical signs included skin lesions (generalized), abrasions (face/neck), decreased activity/lethargic and hunched posture starting at day 9. These clinical signs were attributed secondary to PROJECT 16-1-related immune suppression and opportunistic bacterial infections.

Changes in hematology parameters and clinical chemistry data for early death animals included markedly decreased leukocyte counts (white blood cell count (WBC), neutrophils, lymphocytes, eosinophils, monocytes), decreased red cell parameters (hemoglobin [Hb], hematocrit [Hct], and RBC count) and reticulocyte count, and changes indicative of an acute phase response (decreased albumin, increased globulin and/or fibrinogen). PROJECT 16-1-related changes in flow cytometry parameters (at scheduled sample collection time points prior to necropsy) in these early death animals were similar to those identified for the surviving animals. At the time of unscheduled necropsy, variable changes were present for the monocyte, T-lymphocyte, and NK cell populations. The B-lymphocyte values for all group 4 (3 mg/kg) animals had recovered to prestudy baseline values at the time of unscheduled necropsy.

Macroscopic and microscopic findings indicate that the cause of morbidity and early death in all animals was related to a marked decrease in red blood cell mass, moderate to marked bone marrow hypocellularity (erythroid and myeloid), marked systemic lymphoid depletion characterized by a marked decrease in the number of lymphocytes in the thymus, spleen, mandibular lymph node, mesenteric lymphoid, and GALT (immunosuppression), and severe opportunistic bacterial infections. In the spleen, lymph nodes, and GALT, the diagnosis “decreased number and size, lymphoid follicle/germinal center” was used to further characterize the distribution of the lymphoid depletion. In the bone marrow, erythroid progenitors were decreased in number (hypocellularity) in all early death animals, while myeloid progenitors were often increased in number (hypercellularity) with a shift toward predominance of early progenitors. The left shift was considered to be most likely in response to the increased tissue demand due to secondary infections (primarily bacterial).

There were no other direct test article-related findings. All other microscopic findings in the early death animal were considered to be related to debilitation, inanition, bacterial infection, bacterial septicemia and/or circulatory failure/hypoxia (shock).

In the surviving animals, there were no test article-related changes in body weight, ophthalmology, electrocardiography, blood pressure, or physical examination parameters in any animals. Two Group 4 (3 mg/kg/dose) animals had low food consumption on days 13 and 14, which was likely related to the overall poor health condition caused by marked changes in hematology parameters.

PROJECT 16-1-related clinical signs in surviving Group 3 (1/0.75 mg/kg/dose) and Group 4

(3 mg/kg/dose) animals included skin lesions, moderate swelling on face, abrasions, and/or limited use of limb (which was associated with skin lesions) in individual animals starting Day 9 (similar to unscheduled animals described above). In general, these clinical signs correlated with changes in clinical pathology parameters and were considered secondary to PROJECT 16-1-related immune suppression and opportunistic bacterial infection.

There were no PROJECT 16-1-related clinical signs in Group 2 (0.5 mg/kg/dose) or Group 7

(0.25 mg/kg/dose) animals and no PROJECT 16-2 or MMAE-related clinical signs were present in Group 5 and 6 animals.

There were moderate to marked, adverse PROJECT 16-1-related changes in hematology parameters at 0.5, 1/0.75, and 3 mg/kg/dose that included decreases in indicators of erythrocyte mass (Hb, Hct, and RBC), reticulocytes, and WBC (neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells between days 2-11. In general, these changes were reversed in a majority of animals by days 16-18; however, TK data indicated significant decrease in PROJECT 16-1 exposure post-day 15, which was likely due to ADA formation. At 0.25 mg/kg/dose there were minimal, non-adverse PROJECT 16-1-related decreases in erythrocyte mass and in some leukocytes.

MMAE-related changes in hematology parameters included minimal, non-adverse decreases in RBC mass and moderate decreases in neutrophils.

There were no PROJECT 16-2-related changes in hematology parameters in Group 5 animals.

There were no PROJECT 16-1, PROJECT 16-2 or MMAE-related changes in coagulation and urinalysis parameters. In addition, there were no PROJECT 16-1-related changes in clinical chemistry parameters at 0.25 or 0.5 mg/kg/dose, and no PROJECT 16-2-related changes in Group 5 animals. PROJECT 16-1-related changes in clinical chemistry parameters at 1/0.75 mg/kg/dose and 3 mg/kg animals included minimal decreases in albumin and increases in globulin resulting in decreased albumin:globulin ratio. Similar changes were present in MMAE-treated Group 6 males.

PROJECT 16-1-related, dose-independent, transient, and recoverable decreases were present for all lymphocyte subsets in Groups 2-4 and Group 7 at the 48 hour post-EOI time point. Similar dose-dependent decreases were also present for monocytes at the 48 hour post-EOI time point for Groups 2-4 and Group 7. Overall the greatest decreases, listed in order of magnitude, were present for monocytes, B-lymphocytes, NK Cells, and T lymphocytes. The Group 5 animals that were dosed with PROJECT 16-2 also presented with reductions in the number of lymphocyte subsets and monocyte populations, which generally trended at the same post dose time points; however, these reductions were generally of lesser magnitude as those present for the animals dosed with PROJECT 16-1, and the levels of these reductions were often inconsistent between male and female animals. As noted above the PROJECT 16-2 animals did not have accompanying changes in hematology as described for the PROJECT 16-1 groups which suggest that the changes in lymphocyte subsets for the PROJECT 16-2 animals are weak and may not be biologically significant. The lymphocyte subset and monocyte populations for each dose group generally returned to prestudy values by the next predose time point (i.e., Day 15, Day 29, or Day 43), and remained variable across all subsequent time points.

The differences in the magnitude of depletion between the cell subsets, treated by PROJECT 16-1, were likely due to varying levels of PROJECT 16-1 target expression on each cell population. The prestudy receptor occupancy assessment demonstrated that the B-lymphocyte and Monocyte populations had the greatest expression of the PROJECT 16-1 target molecule (>90%), followed by the NK cells (approximately 45%-68%), and that T lymphocytes had virtually no expression of the PROJECT 16-1 target molecule (<1%). These data correlated with the levels of PROJECT 16-1 binding present at the Day 1, 1 hour post-EOI, at which time the B-lymphocytes and Monocytes presented with the highest level of PROJECT 16-1 binding (approximately 71% to 81% of B-lymphocytes and 62% to 93% of Monocytes), followed by NK cells (approximately 26%-35% of NK cells), and finally the T lymphocytes, which presented with no binding of PROJECT 16-1 at any post dose time point. Collectively, the receptor occupancy and test article binding data demonstrated that the greatest levels of prestudy target expression were directly correlated to the levels of depletion for each cell population.

In this context the magnitude of decreased monocytes and related toxicity observed for cynomolgus monkeys in this study may be over representing what can be expected in humans. Internal data generated at Agensys demonstrates that the percentage of monocytes expressing CD37 in human blood is much lower than the percentage of monocytes expressing the target in monkeys ([Figure 22](#_bookmark81)).

## Figure 22. Immune Cell Populations Determined in Human PBMCs

Following administration of PROJECT 16-1 and PROJECT 16-2 (antibody), the peak serum concentrations were attained within 5 minutes upon IV infusion. Area under the serum concentration-time curves (AUC) and Cmax for PROJECT 16-1 appeared to increase approximately proportionally to dose between 0.25 and 3 mg/kg. The AUC determined using the TAb concentrations was generally larger in comparison to ADC areas due to unconjugation of the ADC over time. Serum concentration of unconjugated MMAE increased gradually after dosing and reached a maximum by 12-24 hours postdose. Serum MMAE concentrations were approximately > 10,000-fold lower than ADC and TAb concentrations, respectively.

Exposure of the antibody (PROJECT 16-2) (Cmax and AUC[0-7]) was similar to the ADC and

TAb at 3 mg/kg dose of PROJECT 16-1. IV injection of the small molecule drug, MMAE, at a molar equivalent dose to the payload of a 3 mg/kg PROJECT 16-1 infusion dose showed that the mean serum MMAE half-life was 0.708 ± 0.0847 and 0.861 ± 0.179 days after the first and last dose, respectively. The T½ z determined for the MMAE drug payload following IV infusion of 3 mg/kg PROJECT 16-1 (T½ z = 2.48  0.375 days) was partially attributed to its formation and was associated with systemic levels of the conjugated ADC (PROJECT 16-1).

Incidences of seroconversion in male and female cynomolgus monkeys were 0%, 70%, 50%, 30%, 60%, and 60% for Dose Groups 1, 2, 3, 4, 5, and 7, respectively.

At terminal euthanasia Day 50, PROJECT 16-1-related microscopic findings were present in the spleen, lymph nodes (mandibular and mesenteric), and GALT and bone marrow at ≥ 1/0.75 mg/kg/dose and kidney at 0.5 mg/kg/dose. Bone marrow findings were characterized by mild myeloid hypocellularity (with a shift towards greater numbers of early myeloid progenitors as compared to late) and minimal erythroid hypercellularity (decreased in the myeloid to erythroid ratio). The bone marrow findings were considered to represent a regenerative/recovery response. In the kidney, moderate glomerulonephritis (increased glomerular mesangium) was identified in one 0.5 mg/kg/dose group male. Based on the nature of the microscopic findings, the glomerular changes were considered to be most likely associated with an immune complex deposition. MMAE-related microscopic findings were

identified in the bone marrow only (0.05 mg/kg/dose). The bone marrow changes were similar in character, yet increased in incidence and severity as compared to those described in the 1/0.75 mg/kg/dose PROJECT 16-1 dose group. There were no PROJECT 16-2-related microscopic findings. All findings recovered after the dose free period.

Following the recovery period for the high dose (3 mg/kg) PROJECT 16-1 treated group, microscopic findings were still present in the spleen, lymph nodes (mandibular and/or mesenteric), and/or GALT in 2 of the 3 animals. Similar to the terminal euthanasia, lymphoid depletion characterized by a decrease in number/size of germinal centers was noted. In 1 animal, a mild increase in protein deposition and lymphoid necrosis (active inflammation and necrosis) was evident in germinal centers. There was no evidence of PROJECT 16-1- related microscopic findings in the other target tissues (thymus, bone marrow, or kidney) after the 49 or 50 day dose-free interval.

At Recovery Euthanasia Day 93, no PROJECT 16-1, PROJECT 16-2 or MMAE-related microscopic findings were noted in the target tissues evaluated (spleen, thymus, lymph nodes [mandibular and mesenteric], GALT, bone marrow [sternum], and kidney).

## Conclusions

In conclusion, administration of PROJECT 16-1 to monkeys at 1 and 3 mg/kg/dose resulted in mortality/morbidity. Macroscopic and microscopic findings in animals that died or were euthanized early indicated that the cause of morbidity and early death in all animals was related to a PROJECT 16-1-related immunosuppression characterized by marked systemic lymphoid depletion, bone marrow suppression characterized by moderate to marked bone marrow hypocellularity, an associated decrease in red blood cell mass, and secondary infection. PROJECT 16-1 at 0.25 and 0.5 mg/kg/dose, the lowered PROJECT 16-1 at 0.75 mg/kg/dose from Day 15, PROJECT 16-2 at 3 mg/kg/dose, and MMAE at 0.05 mg/kg/dose were generally well tolerated. As described above, exposure was variable over the course of the study based on the presence of ADA. Although exposure was generally below the limit of quantitation beyond day 7 for the 0.25 and 0.5 mg//kg groups, some test article related findings were noted for the 0.5 mg/kg dose. No mortality or adverse clinical signs were noted at 0.5 mg/kg. In contrast a single dose of 1 mg/kg was associated with mortality attributed to marked immunosuppression and systemic bacterial infection. Some hematologic changes at this dose were noted as early as day 2 but recovered prior to the next dose. Immune cell populations were also decreased at 0.5 mg/kg but recovered prior to the next dose. Limited microscopic changes were noted at this dose group. Therefore, under the conditions of this study the HNSTD for PROJECT 16-1 is considered to be 0.5 mg/kg.

## Genotoxicity

No genotoxicity studies have been conducted to date with PROJECT 16-1. The 3 genotoxicity studies summarized below were conducted with MMAE and were GLP compliant.

## In Vitro Genotoxicity of MMAE

*Bacterial Reverse Mutation Assay*

An in vitro bacterial reverse mutation assay was performed to evaluate the mutagenic potential of MMAE [Study AA66EH.503.BTL]. MMAE was tested at concentrations of 75, 200, 600, 1800, and 5000 μg per plate using 5 tests strains of bacteria (*Salmonella typhimurium* [TA98, TA100, TA1535, TA1537] and Escherichia coli tester strain [WP2uvrA]) in the presence and absence of Aroclor-induced rat liver S9 enzymes.

Under the conditions of this study, MMAE did not induce mutations in bacteria.

*Mammalian Cell Gene Mutation Assay*

An in vitro assay was conducted to evaluate the ability of MMAE to induce forward mutations at the thymidine kinase locus in the mouse lymphoma L5178Y TK+/- cell line [Study 8204-155]. The assay was conducted in the presence and absence of an exogenous metabolic activation system (S9) and mutagenic potential was assayed by colony growth in the presence of 5-trifluorothymidine resistance (TFTr).

No dose-dependent increase in mutant frequency, or net increase of ≥ 90 TFTr mutants/106 clonable cells above the average concurrent vehicle control mutant frequency were observed with MMAE treatment. Therefore, MMAE was determined to be negative in the L5178Y TK+/- mouse lymphoma forward mutation assay.

## In Vivo Genotoxicity of MMAE

*Micronucleus Assay*

A study was conducted to evaluate the in vivo clastogenic potential and/or the potential for disruption of the mitotic apparatus of MMAE by assessing the formation of micronuclei in polychromatic erythrocyte cells in rat bone marrow following a single IV dose [Study 8204- 151]. Thirty-five male rats were assigned to 5 groups that were administered a single IV dose of the vehicle (0.01 N HCl), cyclophosphamide (positive control) at 60 mg/kg or MMAE at 0.01, 0.1 or 0.2 mg/kg. There were no treatment-related clinical signs of toxicity. However, there was a statistically significant increase (P ≤ 0.01) in the formation of micronucleated polychromatic erythrocytes in bone marrow of animals dosed with MMAE at 0.1 and 0.2 mg/kg.

To investigate the potential mechanism of micronuclei formation, a supplemental study was conducted to evaluate the clastogenic (chromosome breaks) and aneugenic (chromosome lagging) potential of MMAE. The results revealed that MMAE induced predominantly the formation of centromere-positive micronuclei. This is consistent with aneugenic micronuclear formation and the expected microtubular network disrupting mechanism of action of MMAE.

## Carcinogenicity

No carcinogenicity studies have been conducted to date with PROJECT 16-1.

## Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies have been conducted to date with PROJECT 16-1. However, a GLP-compliant study was conducted to evaluate the embryo-fetal development toxicity of MMAE when administered to pregnant rats [Study 8204-397].

Time-mated dams were treated gestational days (GDs) 6 and 13 with vehicle control or MMAE at 0.2 mg/kg via IV bolus injection. Main study dams were euthanized on GD21. Embryo-fetal development toxicity findings were characterized by significant increases in total resorptions, post implantation loss, early delivery, and loss of viable fetuses following treatment with MMAE. These effects on embryo-fetal development are also consistent with the pharmacologic disruption of microtubules caused by MMAE.

MMAE crossed the placenta and was measurable in fetal serum.