Proposal Title –

Phase III Double-blind, Placebo-controlled Study of BXCL701 for the Treatment of Metastatic Castration-Resistant Prostate Cancer in Men

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1.0 Introduction and Background

Prostate cancer is a form of cancer that begins in the gland cells of the prostate, which is found only in the male population. Early stages of prostate cancer rely on testosterone to grow and sometimes, lowering testosterone can control growth. If prostate cancer spreads beyond the prostate, it is called “metastatic,” and is found growing in other organs or tissues. metastatic castration-resistant prostate cancer is more commonly known as mCRPC. It can be difficult to treat. Advanced prostate cancer like this can be life threatening if it spreads to other parts of the body.

Castration-resistant prostate cancer (CRPC) is a form of advanced prostate cancer. With CRPC, the cancer no longer completely responds to treatments that lower testosterone. It shows signs of growth, like a rising PSA (prostate-specific antigen), even with low levels of testosterone. With Metastatic CRPC (mCRPC), the cancer stops responding to hormone treatment, and it is found in other parts of the body. It can spread to nearby lymph nodes, bones, the bladder, rectum, liver, lungs, and maybe the brain.

BXCL701 is an intervention that is orally administered innate immune activator that is designed to initiate inflammation within the tumor microenvironment. The disease area is cancer, and the population is men with metastatic castration-resistant prostate cancer, or mCRPC1. The current

standard of care, pembrolizumab does not address cancers that appear “cold” or uninflamed. BXCL701 is created to render those “cold” tumors as “hot” to make them detectable by adaptive immune systems. Thus, this trial must be conducted because it is targeting an unaddressed research area.

This double-blinded, two parallel group superiority design study evaluates the impact of BXCL701 on mCRPC in male adults. The target population is male adults who are 18 years old or older who are affected by mCRPC. The intervention group will be utilizing both the new intervention, BXCL701, and the current standard of care, pembrolizumab. The patients will receive a fixed-dose of pembrolizumab (200 mg IV q21-days) once orally, along with BXCL701 orally on days 1-14 at recommended Phase 2 dose (RP2D) schedule, which is BXCL701 0.3 mg BID. The time of follow up for each individual patient will be 21 days1. The control group will be utilizing only the current standard of care, pembrolizumab. The patients will receive a fixed-dose of pembrolizumab (200 mg IV q21-days) once orally. The time of follow up for each individual patient will be 21 days.

2.0 Objectives

i) Primary

The primary objective is assessing the composite response rate for the combination of BXCL701 and Pembrolizumab. The time frame is up to 36 months. This will be assessed using the response evaluation criteria for solid tumors includes circulating tumor cell conversion from >5/7.5 mL to

<5/7.5 mL per Veridex assay, along with 50% or greater prostate-specific antigen decline from baseline.

Regarding this primary outcome, the statistical question would be:

Is there a difference between the composite response rates for mCRPC patients who are orally administered BXCL701 in combination with Pembrolizumab and patients who are administrated only Pembrolizumab?

Null hypothesis (H_0) with Alpha = 0.05:

- For patients who orally consume BXCL701 in combination with Pembrolizumab, the mean change of composite response rates from the baseline is the same as the patients who only take Pembrolizumab at the end of the 21-day treatment period.

Alternative hypothesis (H_a) with Alpha = 0.05:

- For patients who orally consume BXCL701 in combination with Pembrolizumab, the mean change of composite response rates from the baseline is *not* the same as the patients who only take Pembrolizumab at the end of the 21-day treatment period.

Direction of clinical interest will be one-sided test to assess if the addition of BXCL701 is superior to the current standard of care by itself. The analysis of primary outcome will utilize a two-sided test to see whether there is any difference between the two group in terms of mean change in composite response rates

ii) Secondary

The secondary objective is assessing the pharmacodynamic profile of BXCL701 and Pembrolizumab. The time frame is up to 36 months.

- Assessed by measuring relevant effects on those cytokines previously shown to be modulated by BXCL701 in humans.

Null hypothesis (H_0) with Alpha = 0.05:

- For patients who orally consume BXCL701 in combination with Pembrolizumab, the pharmacodynamic profile is the same as patients who only take Pembrolizumab at the end of the 21-day treatment period.

Alternative hypothesis (H_a) with Alpha = 0.05:

- For patients who orally consume BXCL701 in combination with Pembrolizumab, the pharmacodynamic profile is *not* the same as patients who only take Pembrolizumab at the end of the 21-day treatment period.

iii) Safety

First: Adverse reactions toward BXCL701, such as hypertension, fatigue, or rashes. This will be measured using the coordinators' judgment daily, and the response will be recorded as categorical answers, specifically YES or NO.

Second: Allergic reactions toward BXCL701. This will be measured using a skin prick test to check for immediate allergic reactions, and the response will be recorded as categorical answers, specifically YES or NO. Data will be collected and assessed by coordinators daily.

Third: Significant increase in stress level. This will be measured using the Perceived Stress Scale, and it will be recorded as continuous variables, specifically a scale from 0 to 10. It will be assessed by coordinators daily.

3.0 Trial Design

i) RCT Features

This trial is a double-blinded, two parallel group superiority design study. It incorporates an achieves the following seven features of an idea RCT as seen below:

Prospective: Longitudinal cohort study conducted over 26 months to see how the two patient groups will react to the new treatment intervention.

Intervention: The intervention is an active treatment, BXCL701, that is administrated by the workers.

Control Group: This trial consists of two groups, with one being the treatment group (active intervention and active standard of care) and one control group (active standard of care).

Randomization: The assignment of patients into the two groups is by chance.

Double blinding: There will be double blinding to ensure that the doctors, their staff, and the patients themselves do not know the interventions to which the patients are assigned to.

Intent-To-Treat Primary Analysis: The patients will be assessed according to the groups they were originally assigned to.

Complete Follow-up: The follow-up period for each patient is 21 days and must be completed after assessment.

ii) Blinding

This study will be a double-dummy scheme. Group A will be randomized to BXCL701 and pembrolizumab. Patients will receive two sets of identical medication bottles. One set contains active BXCL701, and one set contains active pembrolizumab. Group B will be randomized to just pembrolizumab. Patients will receive two sets of identical medication bottles. One set contains placebo BXCL701, and one set contains active pembrolizumab. The double-dummy

scheme is a medication masking system that ensures blinding, specifically blinding the doctors, their staff, and the patients themselves so that they do not know the interventions to which the patients are assigned to.

iii) Randomization

This is a randomized controlled trial, which is known as the best method to prove causality in spite of various limitations. Random allocation is a technique that chooses individuals for treatment groups and control groups entirely by chance with no regard to the will of researchers or patients' condition and preference. This allows researchers to control all known and unknown factors that may affect results in treatment groups and control groups.

iv) Inclusion and Exclusion Criteria

Main inclusion criteria:

1. Patient is 18 years or older.
2. Patient has signed informed consent.
3. Patient can adhere to study visit schedule along with other protocol requirements.
4. Patient has progressive, metastatic castration-resistant disease, as defined by the PCWG3 criteria.
5. Progression during or following completion of at least 1 prior line of systemic therapy for locally advanced or metastatic prostate cancer.
6. Patient has serum testosterone <50 ng/dL during Screening
7. Patient has Eastern Cooperative Oncology Group performance status of 0 to 2.
8. Patient's acute toxic effects of previous anticancer therapy have resolved to ≤Grade 1

9. Patient has adequate baseline organ and hematologic function.
10. Male patients and their female partners must agree and commit to use a barrier contraception throughout the duration of the study until at least 6 months following the last dose of study drug, in addition to their female partners using either an intrauterine device or hormonal contraception and continuing until at least 6 months following the last dose of study drug.

Main exclusion criteria[5]:

1. Patient has received treatment with >2 cytotoxic chemotherapy regimens for castration-resistant prostate cancer (CRPC).
2. Patient has received external-beam radiation or another systemic anticancer therapy within 14 days or 5 half-lives, whichever is shorter, prior to study treatment.
3. Patient has received treatment with an investigational systemic anticancer agent within 14 days prior to study drug administration.
4. Patient has clinically significant cardiovascular disease.
5. QT interval corrected for heart rate using Bazett's formula (QTcB) >480 msec at Screening.
6. Patient has uncontrolled pulmonary disease, symptomatic brain metastases, active autoimmune disease, immunodeficiency, uncontrolled intercurrent illness, human immunodeficiency virus, hepatitis B/C, or any medical condition which, in the opinion of investigator, puts the patient at an unacceptably high risk for toxicity.
7. Patient has known positive status for human immunodeficiency virus, active or chronic Hepatitis B, or Hepatitis C. Screening is not required.

v) Enrolling Centers

Enrolling centers will include research hospitals, medical colleges, research centers, and through telehealth.

vi) Data Coordination and Trial Management

There will be Data Coordinating Center (DCC) and Clinical Trial Management (CTM) resources at a level appropriate for this trial since it is a multi-site trial. The role of the DCC is to ensure quality standard of data management and analysis, provide necessary manuscripts, document, and information for data collection and other aspects of the trial, coordinate and monitor the study. It is responsible for the collection, verification, and storage of all data collected from all the sites that are involved in this multi-site trial. The role of the CTM is to ensure the clinical trials will be completed within time frame, budgets, and desired quality, and to lead the trial activities where necessary. It serves as a single-centralized, web-based enterprise resource to support the clinical research studies conducted across all sites of the trial.

4.0 Data Collection and Patient Follow-up

i) Outcome Details

Primary outcome:

Change from Baseline in Self-Reported Anxiety as Assessed by the Beck Anxiety Inventory (BAI) in 6 weeks.

The BAI is a 21-item self-report measure used to rate subjective, somatic, and panic-related symptoms of anxiety on a scale of 0 to 3, and will be given to participants on a weekly basis.

This instrument accurately reflects the outcome of interest because it is a professionally acknowledged test for anxiety level and have been successfully conducted in the Phase I of this study[7]. The patient will be called on phone for their self-report BAI score when they are not visiting the research site, or they will be asked at their visit for new intervention solutions, which will be dispensed every week. The outcome variable, BAI score, is a continuous variable. The Data Coordinator from DCC will record the participants BAI score.

Secondary outcome:

1) Change from baseline in sleeping quality as assessed by self-reported sleeping quality rate. Participants will rate their sleep quality in a continuous scale from 0 (lowest quality) to 10 (highest quality) during each week in the trial for a total of six weeks. The patient will be called on phone for their self-rated sleeping quality when they are not visiting the research site, or they will be asked at their visit for a new CBD solution. This instrument accurately reflects the outcome of interest because a self-reported score precisely depicts how the patient consider their sleeping quality. Their self-reported score will be recorded by Data Coordinator from DCC.
2) Change from baseline in blood pressure as assessed using a blood pressure monitor. Participants' blood pressure, which is a continuous variable, will be recorded during each week in the trial for a total of six weeks. The patient will be called on phone for their blood pressure if they have one blood pressure monitor at home. If not, the research site will provide

measurement for participants and require them to measure it weekly. This instrument accurately reflects the outcome of interest because increasing blood pressure is a byproduct of increasing anxiety, and a blood pressure monitor is used widely in hospitals and clinics to record blood pressure. Their blood pressure value will be recorded by Data Coordinator from DCC.

3) Change from baseline in School or work performance as assessed by self-reported sleeping performance rate. Participants will rate their School or work performance in a continuous scale from 0 (lowest quality) to 10 (highest quality) during each week in the trial for a total of six weeks. The patient will be called on phone for their self-rated School or work performance when they are not visiting the research site, or they will be asked at their visit for a new CBD solution. This instrument accurately reflects the outcome of interest because a self-reported score precisely depicts how the patient consider their school or work performance. Their self-reported score will be recorded by Data Coordinator from DCC.

ii) Data Collection Mechanism

The mechanism for data collection will utilize a web-based data management system with Electronic Case Report Forms (eCRFs).

iii) Schedule of Visits

Table 1. Schedule of Visits

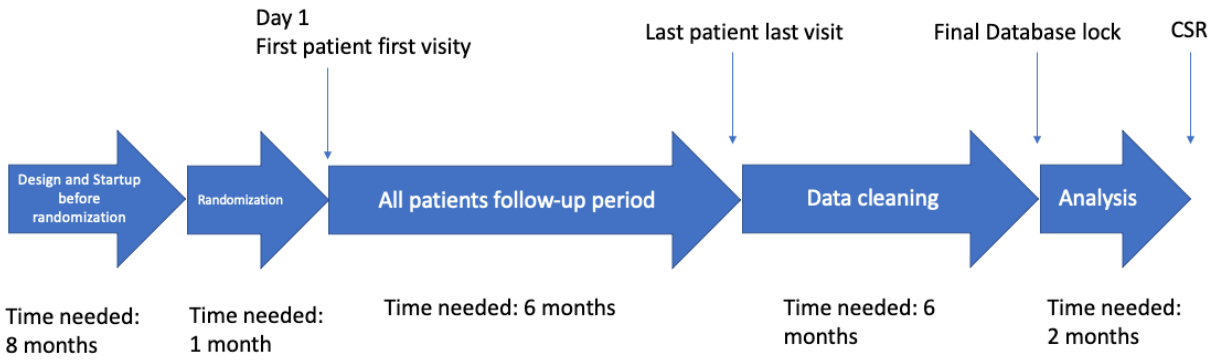
	Screening period		Double0blinded treatment period						
Millstones			Baseline					Last treatment dispenses	EOT EOS
Visit Number	V1	V2	V3	V4	V5	V6	V7	V8	V9
Day	d-14 to d-8	d-7	d1	d8	d15	d22	d29	d36	d43
Week	Wk-2	Wk-1	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
Screening/Baseline:									
Informed Consent									
Inclusion/exclusion									
Medical history									
Demographic									
Randomization									
IVRS/IWRS Contact									
Treatment:									
Administration Training									

Administer study drug									
Study drug dispensation									
Diary check									
Safety assessments:									
BAI score									
Adverse reaction towards intervention solution									
Blood pressure									
Primary outcome assessment:									
Change from baseline in BAI score									
Secondary outcomes assessment:									
Change from baseline in sleeping quality									

Change from baseline in blood pressure									
Change from baseline in school or work performance									

iv) Trial Timeline

Figure 2. Trial Timeline



5.0 Statistical Considerations

i) Type of Outcome

The primary outcome, change from Baseline in Self-Reported Anxiety as Assessed by the Beck Anxiety Inventory (BAI), is a continuous variable. Two-sample T-test assuming equal variance will be utilized.

Let $\mu_I = The\ true\ mean\ of\ change\ from\ baseline\ in\ BAI\ score\ in\ CBD\ solution\ group$

$\mu_c = The\ true\ mean\ of\ change\ from\ baseline\ in\ BAI\ score\ in\ placebo\ solution\ group$

Null hypothesis: $\mu_c - \mu_I = 0$

Alternative hypothesis: $\mu_c - \mu_I \neq 0$

The statistical design is superiority. Type I error of 0.05 and Power of 0.9 is required, and the test is two sided.

ii) Power Calculation:

Unadjusted and Adjusted Effect Size:

According to PASS calculation, unadjusted effect size is 0.8989 using a sample size of 105 for each intervention group and standard deviation of 2, since a previous Phase III study investigated on anxiety and drug treatment utilized a sample size of 210[10]. As such, the calculation of effect size will use 105 for each intervention group

To find out adjusted sample size, assuming for both treatment and control group, %crossover = 5%, %non-compliers = 20%, %full-compliers =75%. Calculation process see appendix 1. The calculated adjusted effect size is 0.63.

A reasonable effect size is clinically defined. It is an important indicator of clinical significance of the results and benefit. Effect size indicates the difference in outcomes between groups. A smaller effect size shows a smaller difference between treatment and control groups. As such, determining the least clinically meaningful effect size is important.

The adjusted effect size, 0.63, is used in the sample size calculation instead of the unadjusted effect size to alleviate effect results from non-compliance and crossover, and to help to give a more reasonable sample size after adjusting for non-compliance and crossover.

iii) Sample Size

A total sample size of 430 (215 per group) is needed to achieve 90% power for an adjusted effect size of 0.63 unit decline in functional outcome, $\mu_I = 1.63,\ \mu_c = 1$, standard deviation = 2, at a significance level of 0.05 two-sided.

PASS output see appendix 2.

iv) Sensitivity Analysis

Table 2. Sensitivity analysis for continuous outcome: varying effect size and standard deviation

Total sample size with power = 0.9			
	$\delta = 0.5$	$\delta = 0.63*$	$\delta = 0.75$
$\sigma = 1$	172	108	76
$\sigma = 2*$	682	430	304
$\sigma = 2.5$	1064	670	474

* Used in adjusted sample size calculation

This table shows how the sample size change as standard deviation and effect size change. Holding effect size fixed, the larger the standard deviation the larger the sample size. Holding standard deviation fixed, the larger the effect size the smaller the sample size. In this proposal, $\delta = 0.63$ and $\sigma = 2$ are used and the adjusted sample size is 430, which is larger than previous study on anxiety and drug treatment[?], but still in acceptable range.

v) Interim Analysis Plan

In the group sequential design, 3 number of looks will be used. Maximum time is 1, and informations are 5,10,15. The methodology used to produce the upper and lower stopping boundaries with correct type 1 error is O'Brien-Fleming method. The type I error will be

controlled appropriately by using smaller level of significance. As such, the null hypothesis will be rejected by a smaller p-value.

Since there is a placebo control intervention in this trial, asymmetrical stopping boundaries will be used. Upper boundaries Z value (corresponding to alpha = 0.025): 3.71020 2.51141 1.99302. Lower boundaries Z value (corresponding to alpha = 0.05): -3.20010 -2.14080 -1.69478.

Figure 3. Upper and Lower boundaries.

Details when Spending = O'Brien-Fleming, N1 = 215, N2 = 215, S1 = 2, S2 = 2, Diff = -0.63

Look	Time	Info	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33333	5	-3.71030	3.71030	0.00021	0.00021	0.00021	0.03402	0.03402
2	0.66667	10	-2.51141	2.51141	0.01202	0.01189	0.01210	0.52797	0.56200
3	1.00000	15	-1.99302	1.99302	0.04626	0.03790	0.05000	0.33892	0.90091

Drift = 3.26599

Details when Spending = O'Brien-Fleming, N1 = 176, N2 = 176, S1 = 2, S2 = 2, Diff = -0.63

Look	Time	Info	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	0.33333	5		3.20010	0.00069	0.00069	0.00069	0.06758	0.06758
2	0.66667	10		2.14080	0.01615	0.01569	0.01637	0.54048	0.60806
3	1.00000	15		1.69478	0.04506	0.03363	0.05000	0.29213	0.90020

Drift = 2.95496

In figure 3, the upper figure shows the upper boundaries, and the figure below shows the lower boundaries but with opposite sign. A negative sign is added to show correct lower boundaries.

6.0 Safety Considerations

i) How each safety outcome of interest from Section 2 iii) is measured.

1) Adverse reaction towards intervention solution, such as allergic. The response will be measured as categorical answer of YES or No. It will be asked on a weekly basis by

coordinator through phone call or when patient visit the research site. In addition, patient should report adverse reaction immediately if they experience one.

2) Major increase in anxiety level. The response will be measured as continuous variable ranging from 0 to 3 (minimal to high anxiety level) using the self-reported BAI test. It will be asked on a weekly basis by coordinator through phone call or when patient visit the research site. A major increase in anxiety level is a score from 0 to 3. In addition, patient should report to the researchers immediately if they think they have experienced abnormal increase in anxiety.

3) Major increase in blood pressure. The response will be measured using a blood pressure monitor. The measurement will be taken on a weekly basis by coordinator through phone call if the patient self-measured, or when patient visit the research site if they want to be measured by researchers. An increase in systolic blood pressure of greater than 20 mm Hg and diastolic blood pressure of greater than 10 mm Hg is considered abnormal and should stop the trial[12].

ii) Reasons why these specific key safety outcomes are important

The first safety outcome, adverse reaction towards intervention solution, is important because this is detrimental to the health of patients. It is not allowed to harm patients in anyway during the trial.

The second safety outcome, major increase in anxiety level, is important for the same reason as the first safety outcome, which is because a major increase in anxiety harm the health of patients and would be unethical to continue the trial even though anxiety is the primary outcome this trial measures.

The third safety outcome, major increase in blood pressure, is important because this is a crucial indicator of increased anxiety. A significant increase in blood pressure also harm patients' health.

iii) Other safety outcomes this trial monitor

This trial also monitors sleeping quality and school or work performance of participants. A major change in these two qualities could be an indicator of abnormal reaction towards treatment. Researchers will examine if a major change in these two qualities is caused by the treatment or other event happened in participants' daily life.

7.0 Limitations and late-breaking problems

The study results may have biases caused by self-reporting. The primary outcome measurement, BAI score (calculated by a 21-item self-report measure), and secondary outcomes measurements, sleeping quality and school or work performance rate are all self-reported by participants. As such, the correct responses might be altered by issues such as recalling error, exaggeration or under-estimation, and misinterpretation of the question. To alleviate this issue, researchers should carefully frame the question to make it not misleading. Researcher may also provide examples regarding to specific questions to make participants clearly understand and give precise answer.

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13.

9. Appendix:

Appendix 1: adjusted effect size calculation

$Unadjusted\ effect\ size\ \delta = 0.8989$

Therefore, $\mu_I - \mu_c = 0.8989$

Let $\mu_I = 1.8989,\ \mu_c = 1, since\ the\ BAI\ score\ ranges\ from\ 0\ to\ 3.$

For intervention group, adjusted mean outcome is:

$$1 * 0.05 + 0.2 * \frac{1+1.8989}{2} + 0.75 * 1.8989 = 1.76$$

For intervention group, adjusted mean outcome is:

$$1.8989 * 0.05 + 0.2 * \frac{1+1.8989}{2} + 0.75 * 1 = 1.13$$

Adjusted effect size = 1.76 – 1.13 = 0.63

Appendix 2: sample size calculation using group sequential tests for two means

Remove time column and correct the information values for the number of cumulative patients at each look