LBURGINAZE™

Engineered Human Arginase 1 for the Treatment of Liver cancer

Techno-Commercial Proposal

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LBURGINAZE™ and its potential to revolutionize the treatment of Liver cancer

Liver cancer is one of the most common malignancies and is a 2nd major cause of cancer mortality globally (accounting for ~9 lakhs cancer deaths annually). Of all liver cancer cases, >90% are hepatocellular carcinomas (HCCs). Treatment strategies include chemotherapy, radiotherapy, immunotherapy and surgery. Immunotherapy and transplantation are more effective than others but these treatments are neither available nor affordable for everyone in our country. Thus, chemotherapy is mostly used in India for the treatment of advanced HCC cases. However, these drugs even at their maximum dose extend the median overall survival of liver cancer patients by ONLY 3-6 months and also possess serious toxicity. Thus, there is a dire need for the development of new strategy for the treatment and management of Liver cancer.

LBURGINAZE™ is an engineered human arginase 1 (rhArg 1) enzyme which not only exhibits anti-cancer activities against a variety of cancers (broad spectrum) but also possesses improved pharmacokinetic properties. Currently, it is in pre-clinical developmental stage.

Current market of liver cancer medication:

The liver cancer medication market is projected to become an active ground for competition in the coming few years. According to Data Bridge Market Research analysis, the expected CAGR of the liver cancer (HCC) drugs market is ~17.61% and it will grow up to USD 14.18 billion by 2030.

Problem statement:

Hepatocellular Carcinoma (HCC) is a leading cause of cancer deaths worldwide, with current treatments like chemotherapy (Sorafenib) offering limited efficacy and high toxicity. Many patients face poor survival rates and significant side effects, underscoring the urgent need for more effective, less toxic therapies. Liver cancer cells' dependence on external arginine due to arginine auxotrophy presents a therapeutic opportunity. Therefore, novel strategies, such as LBURGINAZE™, are needed to improve outcomes for HCC patients.

Solution: LBURGINAZE™

LBURGINAZE is an engineered human arginase 1 that hydrolyzes arginine and exhibit anti-cancer effects. It comprise of native human arginase 1 enzyme linked to a half-life extension partner *via* a flexible peptide linker. LBURGINAZE attenuate cancer in animal model of liver cancer.

Development of LBURGINAZE™

1. ***Designing and engineering of LBURGINAZE variants***: Using protein engineering approaches, we have engineered LBURGINAZE variants.

2. ***Clone development***: Clones (*Pichia pastoris*) capable of producing LBURGINAZE™ variants are developed.

3. ***Production process development***: A simple-n-cost effective process (lab-scale) to produce LBURGINAZE™ variants is developed. This process is amenable for the development of process for the industrial scale production of LBURGINAZE™.

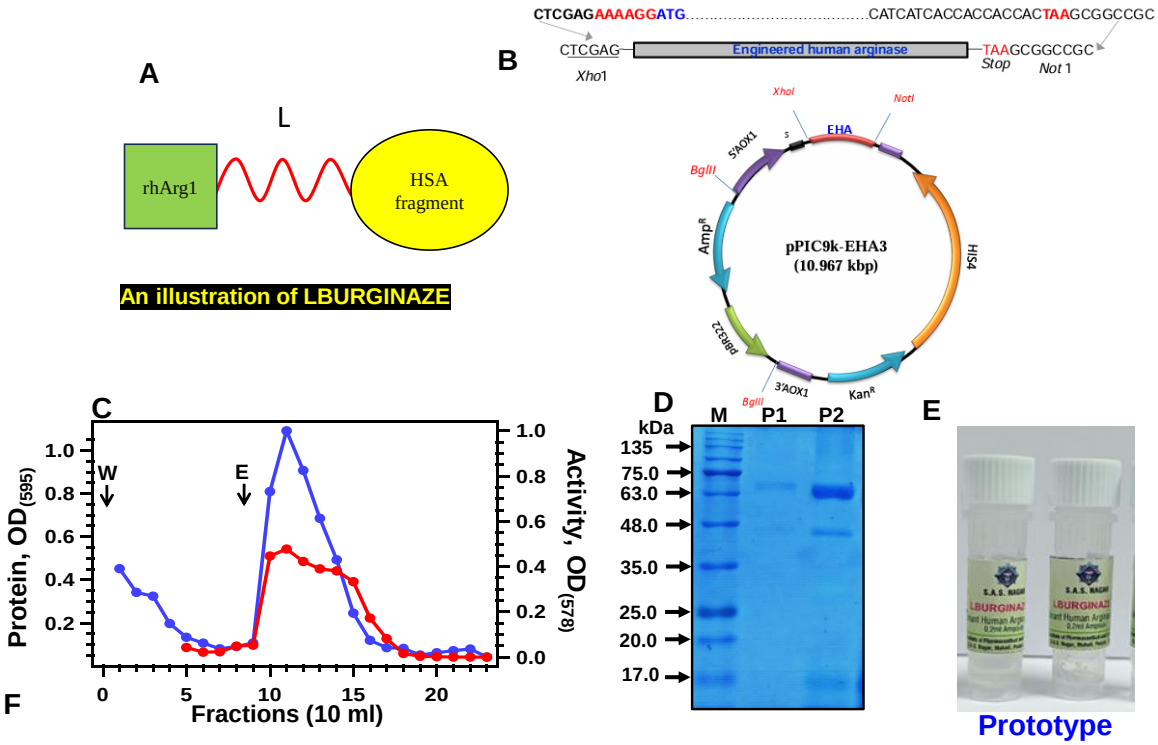


Fig.1 Production of LBURGINAZE: Panel A is design of LBURGINAZE. **Panel B** is a cartoon of expression vector. **Panel C & D** shows chromatogram and SDS-PAGE, respectively, of a typical purification experiment. **Panel E** shows prototype.

4. ***In vitro studies***: Functional characterization of LBURGINAZE™ variants has been completed using non-cellular and cellular assays. LBURGINAZE has shown a broad spectrum of activity against various types of cancer.

5. ***Efficacy study in animal model of liver cancer***: The efficacy of LBURGINAZE™ variants has already been demonstrated in xenograft model of liver cancer (in the lab of Prof. Kulbhushan Tikoo, Dept. of Pharmacology Toxicology, NIPER SAS Nagar).

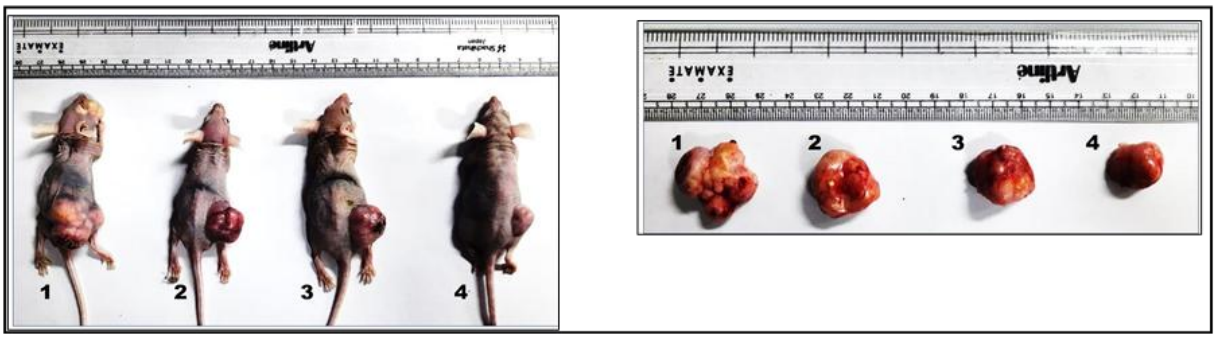


Fig 2. Anti-cancer activity of LBURGINAZE LBURGINAZE combined with 5-fluorouracil (5-FU) was found to be more effective than either 5-FU or LBURGINAZE alone in reducing the growth and prolonging the survival of tumour-bearing animals.

Our Publications:

Prasad, Y. R., Anakha, J., Jawalekar, S. S., & Pande, A. H. (2024). Broad-spectrum anti-cancer activity of fused human arginase variants. **Investigational New Drugs**, 10.1007/s10637-024-01466-8. Advance online publication. https://doi.org/10.1007/s10637-024-01466-8

Prasad, Y. R., Anakha, J., & Pande, A. H. (2024). Treating liver cancer through arginine depletion. **Drug Discovery Today**, 29(4), 103940. https://doi.org/10.1016/j.drudis.2024.103940

Jawalekar, S. S., Kawathe, P. S., Sharma, N., Anakha, J., Tikoo, K., & Pande, A. H. (2023). Development and characterization of fused human arginase I for cancer therapy. **Investigational New Drugs**, 41(5), 652–663. https://doi.org/10.1007/s10637-023-01387-y

Anakha, J., Prasad, Y. R., Sharma, N., & Pande, A. H. (2023). Human arginase I: a potential broad-spectrum anti-cancer agent. **3 Biotech**, 13(5), 159. https://doi.org/10.1007/s13205-023-03590-3

Anakha, J., Kawathe, P. S., Datta, S., Jawalekar, S. S., Banerjee, U. C., & Pande, A. H. (2022). Human arginase 1, a Jack of all trades?. **3 Biotech**, 12(10), 264. https://doi.org/10.1007/s13205-022-03326-9

Indian Patent application # 202111011642 Snehal Jawalkar, Kulbhushan Tikoo Abhay H. Pande. Engineered arginase constructs, method of generation and uses thereof.

Indian Patent application # 202211013970 Abhay Hariram Pande, Snehal Sainath Jawalekar, Priyanka Sugriv Kawathe, Nisha Sharma, Kulbhushan Tikoo. Engineered arginase, method of generation and uses thereof.

PCT/IN2022/050258 Snehal Jawalkar, Kulbhushan Tikoo Abhay H. Pande. Engineered arginase constructs method of generation and uses thereof.

Market Opportunity:

There is a growing need for the development of more effective treatment(s) for liver cancer cases. The liver cancer medication market size is projected to grow up to USD 14.18 billion by 2030 with CAGR of ~17.61%. There is a very-high chance that LBURGINAZE™ variants can turn out to be potential blockbusters in the treatment of multiple cancer conditions in coming years!!!

So, there is a HUGE market potential for is LBURGINAZE™ as it can be used for various arginine auxotrophic cancers.

Development Plan: Long term (2025-2035)

Long-term (2025-2035) and immediate short-term (2025-2027) plans for the development of LBURGINAZE™ is given as **Annexure 1**

Financial Projections:

- Cost of non-GLP studies is tabulated in **Annexure 2**

- Further cost will require in-depth discussion with the partner company

Why LBURGINAZE is ideal for liver cancer ?

The current treatment strategies available for liver cancer patients are either non-affordable or are less effective and more toxic. LBURGINAZE can be an ideal candidate for the treatment of liver cancer patients.

- ✓ LBURGINAZE is specifically designed to target the metabolic vulnerabilities of cancer, making it a ***highly efficacious*** treatment option
- ✓ LBURGINAZE is engineered to have improved drug-like properties (pharmacokinetics) making it a safer option
- ✓ ***Cost-effectiveness*** and potential for ***affordability,*** compared to existing treatments, makes LBURGINAZE an ideal candidate for liver cancer patients, particularly in regions like India.

Annexure 1
LBURGINAZE Development Plan -1 (Long term, 2025-2035)

Developmental Stages	Time-line	Milestones
Stage 1: Discovery & Early Development (Already Done) Engineering, clone development, lab-scale production process development; *in vitro* testing and efficacy assessment in animal model of liver cancer is completed. Prototype of product and proof-of-concept is ready.	2014-2024	Prototype, process of production & PoC READY
Stage 2: Non-GLP studies-1 i) Clone optimization and Scale-up of production process (5-10 Liters) ii) Development of stable formulation of LBURGINAZE and stability studies iii) Toxicology studies of LBURGINAZE formulation iv) Safety pharmacology studies of LBURGINAZE formulation v) PK studies of LBURGINAZE formulation		
Stage 3: Non-GLP studies-2 i) Re-validation of stage-2 studies in higher animals ii) Efficacy studies (dose, route, combination etc) of LBURGINAZE formulation in relevant animal model ii) Re-validation of efficacy of LBURGINAZE formulation in relevant animal model		
Stage 4: GLP studies i) GLP-studies for IND application filing ii) IND application filing iii) Rebuttal		
Stage 5: Manufacturing process for clinical studies i) Preparation of LBURGINAZE batch in GMP facility for clinical studies		

Stage 6: Clinical studies i) Phase I Clinical Trials ii) Phase II Clinical Trials iii) Phase III Clinical Trials		
Stage 7: Regulatory Review and Approval i) NDA / BLA filing ii) Rebuttal iii) Product labelling and marketing approvals iv) Post approval preparation and preparation of product launch		
Stage 8: Commercialization and Post-Market Activities i) Product launch ii) Expansion and Scaling iii) Post-Market Surveillance & Lifecycle Management	---	

Annexure 2

LBURGINAZE Development Plan-2 (Jan 2025-Dec 2027)

(Stage 2: Non-GLP studies-1)

Target: to generate data for Stage 3 and 4 studies!

	Experiments	Deliverables	Time-line	Cost
1	**Production**: i) Clone optimization; ii) Process optimization (5 lts fermenter)	- high-yield clone; - optimized process; - data for pilot scale-up (50-100 lts); data for stage-5 experiments	**03 - 18 Mo**	
2	**Formulation & Stability studies**: (as per NDCT-2019 rules) i) Formulation ii) Stability studies iii) Data of final formulation	- 2 final formulations; - stability data; - analytical data of formulation; - data for further pre-clinical/clinical studies as well as for scale-up of formulation	**03 - 18 Mo**	
3	**Toxicological studies**: (as per NDCT-2019 rules) i) *Systemic Toxicity studies* (Single dose / dose ranging toxicity studies; *Repeated-dose systemic toxicity studies* (14/28/90/180 days); ii) *Immunogenicity / Hypersensitivity* studies iii) *Local toxicity studies* with proposed route of application? iv) *Genotoxicity studies* v) *Reproductive toxicology studies* (Male fertility, other studies)	- toxicological profile of formulation - therapeutic index of formulation - data for Stage-3/4 studies	**09 - 33 Mo**	
4	**Safety Pharmacological studies**: (as per NDCT-2019 rules) i) Cardiovascular system ii) Central nervous system iii) Respiratory system	- safety profile of formulation - data for Stage-3 experiments	**12 - 18 Mo**	
5	**Pharmacokinetic studies**: i) All ADME parameters	- Pharmacokinetic data / metabolic profile of formulation - data for Stage-3/4/6	**12 - 18 Mo**	
6	**Administrative cost**			

*Tentative, based on the estimates received.

Funding:

1. Partner company shall fund 100% of these studies, or
2. Partner company & NIPER SAS Nagar together shall arrange funding from various schemes of GoI (ex, BIRAC, ICMR, DST, Others).

SWOT Analysis

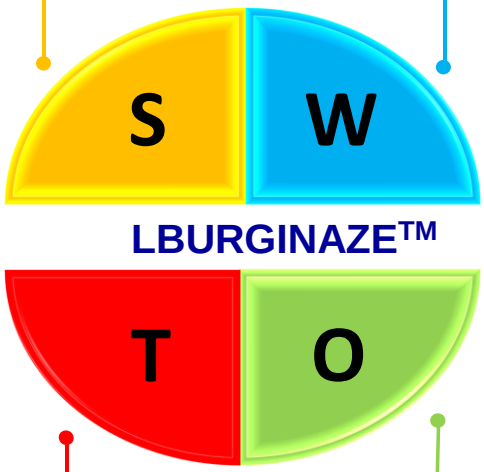
LBURGINAZE™

STRENGTHS

- Anti-cancer activity
- Can inhibit multiple cancer
- Improved Pharmacokinetic properties ($t_{1/2}$)
- Industrial feasible protein production technology

WEAKNESS

- *Pichia* production system
- Detailed toxicity, safety and efficacy studies NOT completed yet



THREATS

- IND filing and clinical studies may take substantial time
- Competitors may GO ahead

OPPORTUNITIES

- Novel anti-cancer biologics for numerous cancer
- Made in India

LBURGINAZE™: Engineered Human Arginase 1 for the Treatment of Liver cancer

1. Field:
Liver cancer, the sixth most common cancer globally and the second-leading cause of cancer-related deaths, presents a critical public health threat. Diagnosis often occurs in advanced stages of the disease, aligning incidence with fatality rates.

2. Problem:
There are various treatment strategies available for liver cancer, including surgery, transplantation, and radiotherapy, which are typically used for early-stage cases. For intermediate and advanced stages, chemotherapy is commonly administered; however, it is associated with serious toxicity issues. Even at maximum doses, chemotherapy extends median survival by only 3-6 months. Thus, there is an urgent need to develop more effective agent(s) for the treatment and management of liver cancer.

3. Need of the hour:
The urgent need for safer, more effective treatments for Hepatocellular Carcinoma (HCC) is critical due to the high mortality and severe side effects of current therapies.

4. Our solution: LBURGINAZE™, an engineered human arginase 1, is an effective anti-cancer biologic.

PATENT STATUS:

Applied

TRL STATUS:
TRL3/4

LBURGINAZE

SPECIFICATIONS OF TECHNOLOGY:

1. **Fusion Protein Engineering**: LBURGINAZE is an engineered human arginase 1 involved in arginine deprivation and manages liver cancer effectively.

2. **Simple-n-cost effective production platform**: High yield clone (*P pastoris*) and simple production process

3. **Superior efficacy**: LBURGINAZE has potential against broad spectrum of arginine auxotrophic cancers.

4. **Protected intellectual property**: Indian and International patent filed.

5. **Made In India !!!**

Please Contact:

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SCAN QR TO SEE THE LAB TECHNOLOGIES

Business Model Canvas for LBURGINAZE™

<table>
<tr>
<td>Problem:

- Liver cancer is a critical public health threat (6th most common cancer globally & 2nd leading cause of cancer-related deaths)
- Rate of liver cancer cases is growing rapidly in low- and middle-income countries
- There is no effective way to prevent or treat liver cancer

Shortcomings:
- Current treatments are inadequate or non-affordable
- A dire need to develop more efficacious treatment</td>
<td>Solution:

- LBURGINAZE™, a engineered human arginase 1 enzyme that hydrolyse arginine in the extracellular body pool and manage arginine-auxotrophic cancers more effectively</td>
<td>Unique Value Prop:

Reduced Adverse Reaction
potential for lower doses & fewer side effects compared to existing treatments)

Cost-Effective
potentially lower production costs, more accessible and more affordable for Indian patients; Made in India

Versatility
potential applicability to a range of cancers and arginase deficiency</td>
<td>Unfair Advantage:

Reduced Adverse Reaction
Potential for lower doses and reduced side effects

Versatility:
Potential applicability for the treatment and management of multiple cancer conditions (~20)

Affordable-n-accessible:
Potential low cost and made in India</td>
<td>Customer Segments:

Disease segments:

Multiple cancers

Partners:

National and international Start-ups & Pharmaceutical companies for co-development</td>
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<td>Existing Alternatives:

Various treatment available for cancer (including surgery, radiotherapy & chemotherapy) are less effective and toxic</td>
<td>Key Metrics:

- Efficacy in multiple pre-clinical models

- Cost of GLP mode proof</td>
<td>High-level Concept:

- Unique design features of LBURGINAZE™ make it a potential candidate for the treatment and management of multiple arginine-auxotrophic cancers</td>
<td>Channels:

National & international
- Start-ups
- Pharmaceutical companies</td>
<td>Early Adopters:

National & international
- Start-ups</td>
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<tr>
<td colspan="3">Cost Structure:

- Cost of non-GLP studies is tabulated in Annexure 2
- Further cost will require in-depth discussion</td>
<td colspan="2">Revenue Streams:

- Funding from government / non-government research grants</td>
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