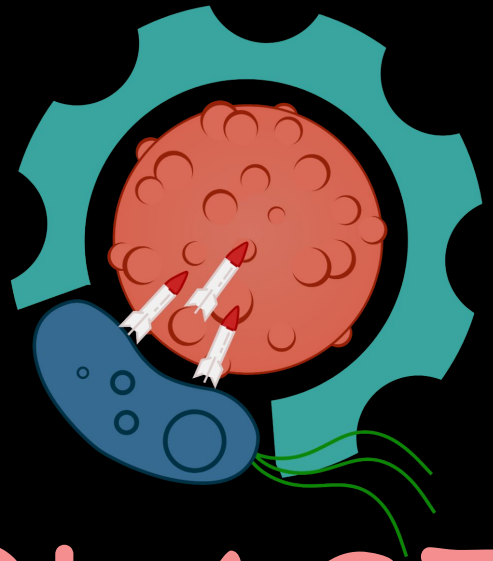
iGEM IISER Bhopal presents

Treatment of Cancer



BLAST

Bifidobacterium longum induced Apoptosis of Cancer cells using Smac and Trail

Inspiration

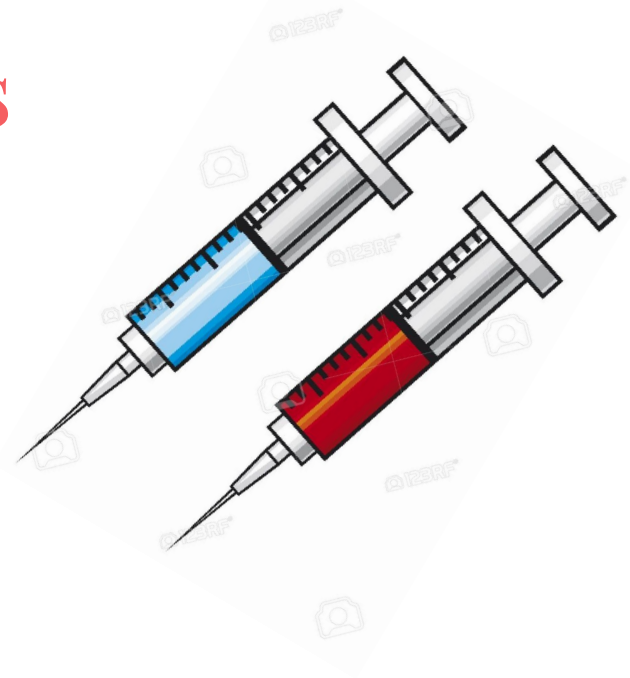
- Cancer is the second leading cause of death worldwide, accounting for 14% of total deaths
- Solid Tumours Constitutes 90 % of all adult human cancers.
- 19.5 million new cases and nearly 10 million deaths reported in 2020 due to cancer.
- The average total cost of cancer treatments is around $150,000

1 out of 10 indians will get affected in their lifetime

Current Solutions

Current Solutions and their Side Effects

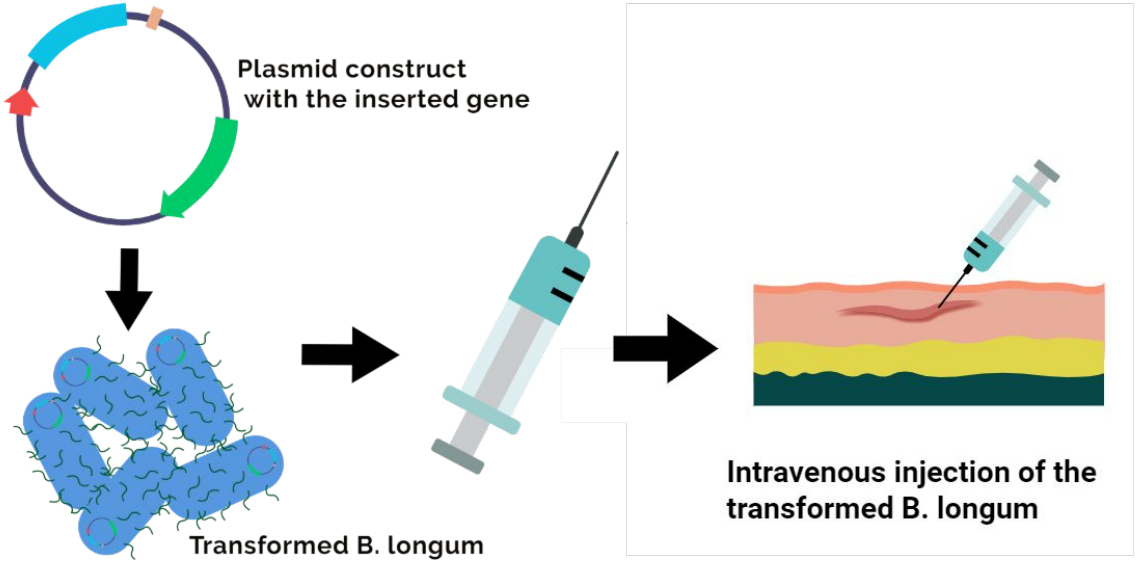
- Chemotherapy (hair loss, nausea, etc.)
- Surgery (pain, damage to nearby tissues, tumor regrowth)
- Radiation therapy (itchiness, skin damage)
- Hormone therapy (causes heart disease, disturbes sex life, blood clotting)



Treatment of cancer is both physically and mentally traumatising. We are proposing a novel, frugal and targeted bacteria-based therapy for solid tumours, presenting a significant advantage over currently available treatments.

Our Solution

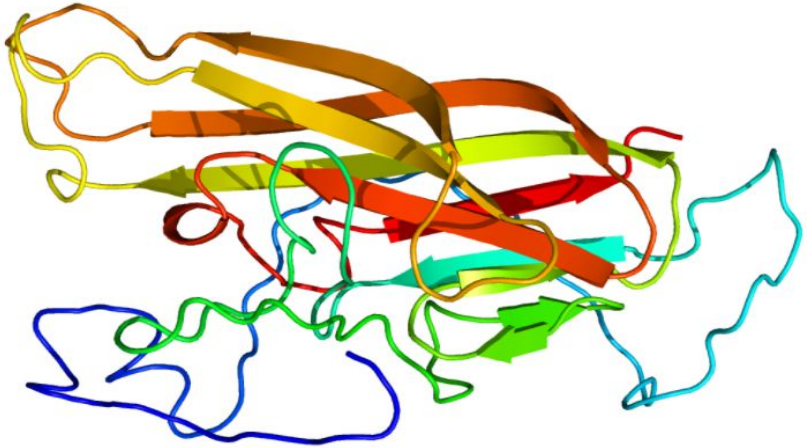
- Bacterial transformation with our plasmid construct pMB1-TRAIL-DIABLO, which will contain TRAIL gene and smac/DIABLO gene.
- Intravenous injection of recombinant Bifidobacterium longum .
- Bacterium being obligate anaerobe localises and induce apoptosis in solid tumors.

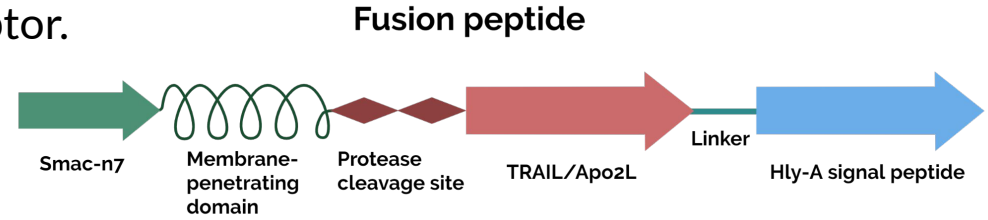


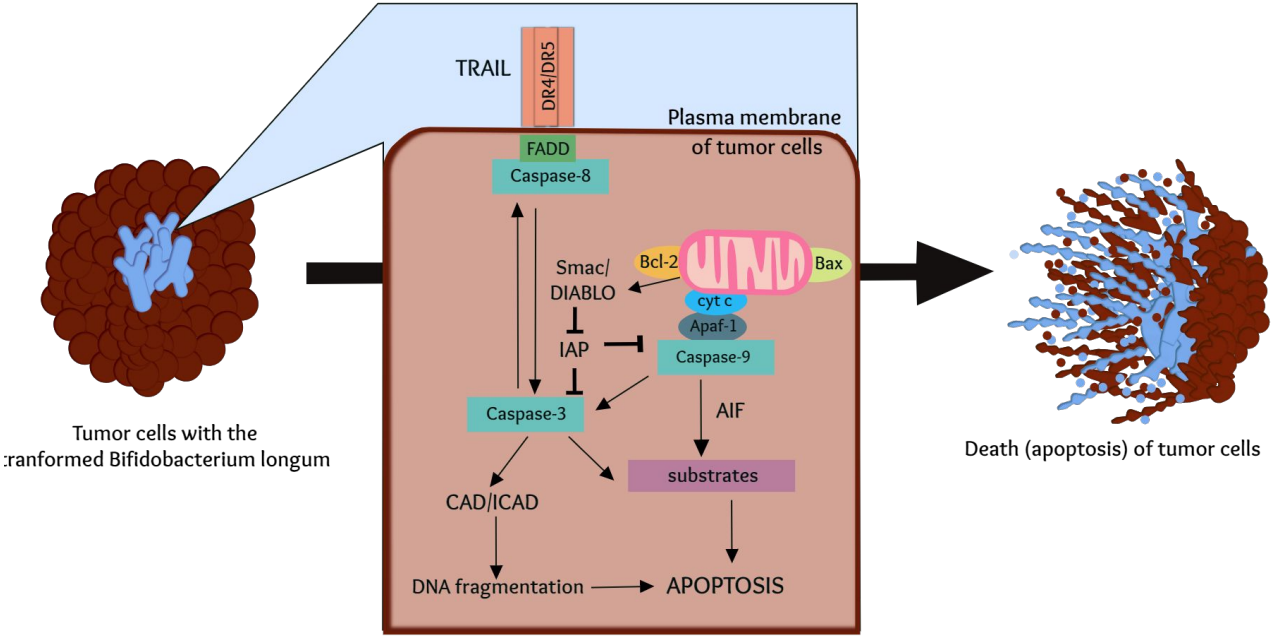
Sasaki T; Fujimori M; Hamaji Y; Hama Y; Ito K; Amano J; Taniguchi S; "Genetically Engineered Bifidobacterium Longum for Tumor-Targeting Enzyme-Prodrug Therapy of Autochthonous Mammary Tumors in Rats." Cancer Science, U.S. National Library of Medicine

Fusion Construct: Smac n7 + TRAIL

- More Robust and efficient than individual TRAIL and Smac
- Kills TRAIL -resistant cancer cells
- Selective Cytotoxicity to tumor cells
- Smac is given a cell penetrating Poly arginine (8R) for internalization
- Protease cleavage and release of Smac take place when TRAIL binds to the Receptor.

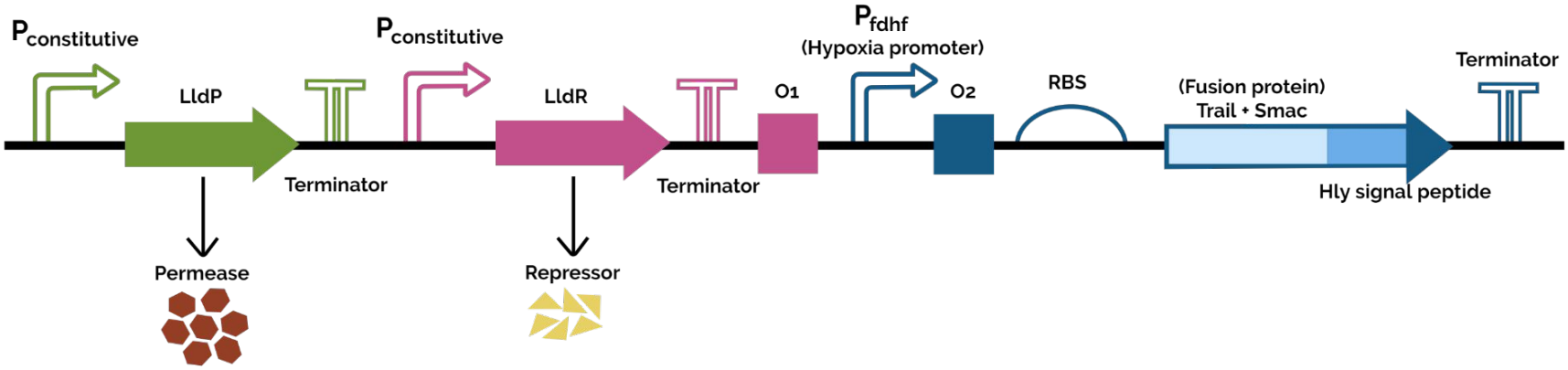






TRAIL released from *B.longum* binds to the TRAIL receptors and initiates apoptosis by recruiting FADD which binds to Caspase-8 to form a complex called **DISC (**Death Inducing Signalling Complex**).** DISC activates Caspase 8 which in turn activates Caspase 3 via proteolytic cleavage. Caspase 3 activates CAD (caspase-activated DNAse) which executes **nuclear apoptotic DNA fragmentation** that causes cell death (Apoptosis). Smac/DIABLO binds to XIAP (thereby inactivating the inhibitor) and also causes apoptosis by intrinsic pathway promoting cleavage and activation of caspase 3.

Gene Construct

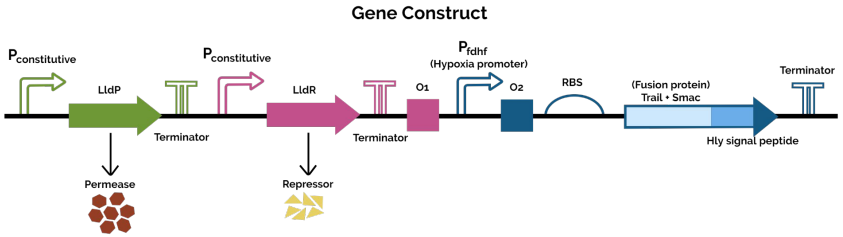


- LldP- Permease -> Allows efficient lactate entry
- LldR -Lactate inducible Repressor
- O1,O2 - LldR sensitive Operators
- fdhF Promoter - Hypoxia inducible promoter
- Hly A -> C terminal Signal peptide

Biosafety

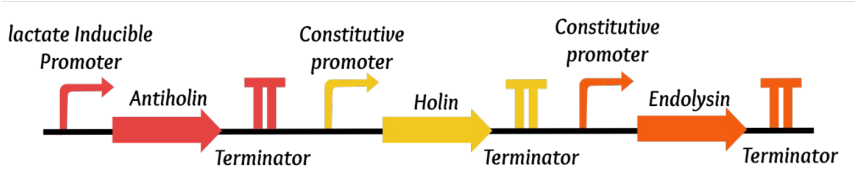
AND Gate :

1. Activated by Lactate and Hypoxia
2. Secretion specificity (secrete proteins only in tumour microenvironment)



KILL Switch :

1. Holin - antiholin / toxin - antitoxin system
2. ensures that the bacteria can survive only in the tumour site where high lactate concentration is present



Holin - Antiholin kill switch

AND Gate functioning

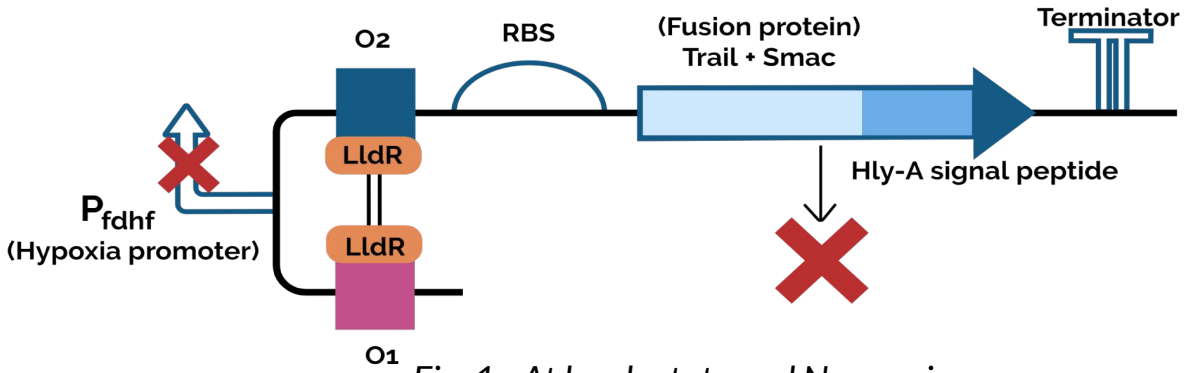


Fig :1 - At low lactate and Normoxia

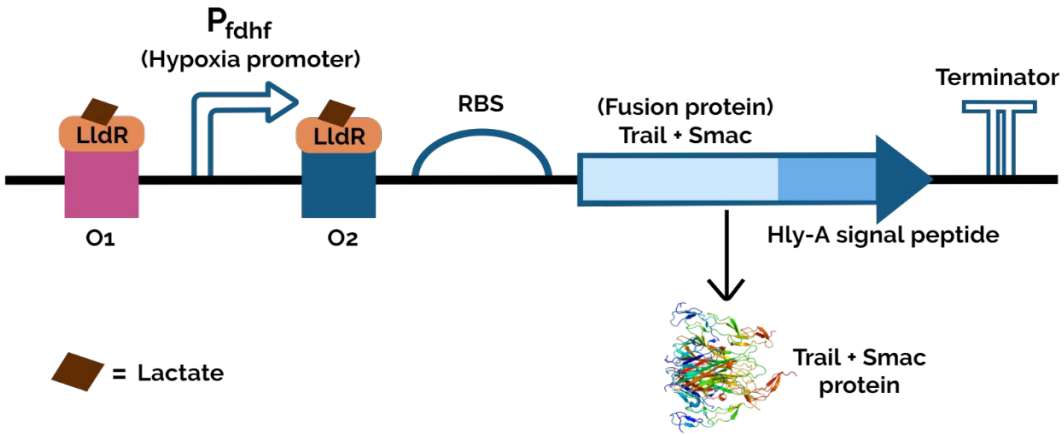


Fig :2 - At high lactate and Hypoxia

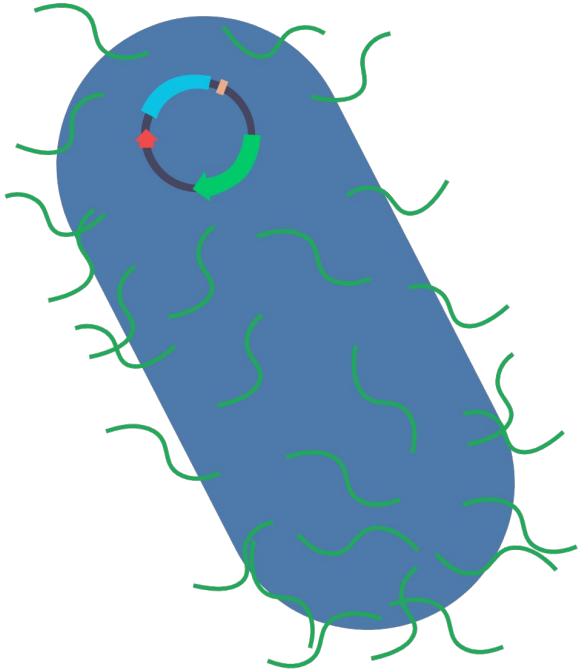
Experimental Protocol

- **GENES-**

  Human-TRAIL gene, Smac/DIABLO gene, AND gate (Hypoxia promoter + lactate operator) and KILL switch.

- **Plasmid construct**-

  Insert construct (TRAIL+ Smac with signal peptide) under lactate operated Hypoxia induced promoter (P fdhF)and the Kill Switch in pMB1.



Transformed B. longum

- **<u>Transformation of B.longum</u>**-

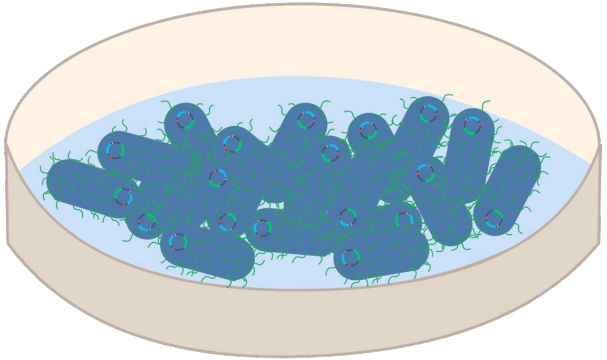
  with plasmid construct pMB1-TRAIL-DIABLO by electroporation.

- **<u>Selection of the transformants -</u>**

  by plating the bacteria on TPY -agar plates with antibiotic spectinomycin resistance in anaerobic conditions.

- **<u>Quantification of the bacteria-</u>**

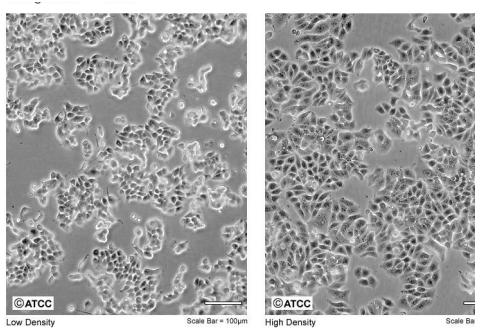
  Inoculation into fresh TPY medium and growth quantified by measuring the optical density (0.4 - 0.6 is considered ideal)



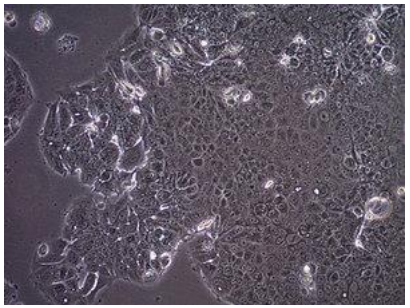
Quantification of bacteria

- **<u>Cell lines and models-</u>**

  MCF7 or T47D ,NCI-H460, A549 ,SK-HEP-1 cancer cell lines .



T47D



MCF7

- **Introducing bacteria** - via intravenous administration in case of xenografts, via direct administering in the medium for cancer cell lines.

- **Analysis of Apoptotic potential** - by apoptosis assay through flow cytometry using Annexin V staining. MTT Assay to analyse cytotoxicity of TRAIL -Smac fusion protein. Caspase-3 checked in tumours via immunoblotting using anti-caspase-3 antibodies

Thank You

Suggestions

1. **<u>To ensure the secretion of TRAIL/ SMAC to be able to bind and induce apoptosis:-</u>**

- **Addition of export signal peptides for both TRAIL and Smac/DIABLO .**

  TRAIL-ompC signal peptide and smac-n7+octa-Arg signal peptide

- **We ran the protein sequence in TOPCONS..**

  Both results showed the presence of export signal peptide and absence of transmembrane domain which shows that the proteins will get secreted.

Suggestions

2. Primary feasibility in UG Lab:-

- Formation of Bacterial culture
- Cloning of plasmids
- Selection of transformants & all other work before in-vitro testing can be done in UG lab.

In-vitro testing on model cell lines to be done in a BSL-2 culture facility.

Suggestions

3. Bio-safety:-

- Lab Safety (ISBC approvals for BSL-1 and BSL-2 safety levels for labs)
- Product safety

Product safety:-

- Bacteria being obligate anaerobe grows only in anaerobic conditions
- Additionally, a kill switch is added to restrict the growth of bacteria in non-tumor regions.
- After doing the required work, bacteria will self destruct in body by phagocytosis.

To further ensure the bio-safety (IHP work):-

- We can talk to the researchers working in the field of breast cancer.
- Consulting Oncologists and other cancer surgeons.

Ethical Approval:- Will submit a design for in-vivo experiment to IEC, once done with in vitro.

Human practises/ IHP:-

1. Read and discuss the new National Guidelines for “Gene Therapy Product Development and Clinical Trials” 2019 released by ICMR. Ensure that our project is following the guideline. Reaching out to the relevant scientists and policymakers. (Ethics)
2. Discuss and validate the project, ideally with a member of the Gene Therapy Research Task-force set up by the ICMR.
3. Enquire about the current status of the District Cancer Control Programme (under NCCP) at our local Government hospital. Under DCCP, a cluster of 2-3 districts is taken up for prevention, early detection, minimal treatment and provision of supportive cancer care at district levels.
4. Contact eminent researchers from Advanced Centre for Treatment Research and Education in Cancer Mumbai, AIIMS Delhi, National Institute of Cancer Prevention & Research, Noida, JNCHRC Bhopal et cetera.

HP/IHP (contd.):-

1. Contact doctors working in cancer treatment to know about new therapeutic techniques, problems and benefits of the current methods in use for cancer treatment.
2. Survey the breast cancer patients and survivors to know and appreciate their journey- emotional, social and financial (Can be contacted through Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal)
3. Survey the community members and people of Bhopal to gauge current levels of awareness on Breast Cancer.
4. Conduct extensive outreach in schools, colleges and the public spaces(malls, parks) to raise awareness and empathy.
5. Conduct fund-raising activities and donate them to relevant organisations.
6. Speak to NGOs working in the field to understand the problems faced at the ground level. (Cancer Foundation India)

HP/IHP (so far):-

Dr. Neetu Kalra :- Suggested the use of cell lines, their availability and about unavailability of BSL-2 culture facilities .

Dr. Sudeesh AP *(Postdoctoral Associate- The Jackson laboratory of Mammalian Genetics, Bar Harbor, U.S.)* :- Helped with plasmid construct and the use of various lab proto

Medal Criterion for iGEM

Attendance

Competition deliverables:- wiki, poster ,presentation &promotion videos

Attributions from members

Contribution:- Hardware(Fluoroscence reader), and new bio bricks to igem

Engineering success:- designing all parts, implementation & data analysis

Collaboration:- As mentioned can be done witheither Team cornell or any other current iGem team

HP:- getting ground level report based on govt. plans, with the help of NGOs, engaging with all the stakeholders related to breast cancer

Modelling:- Caspase -3 apoptopic modelling, tumour growth modelling & modelling of m- cardinal can be done for the kinetic data through MATLAB/ PYTHON scripts

IHP:- Corrections/enhancement in project based on HP practises

Documentation of improved registry parts, Extensive science comm with schools, colleges opening a blog for cancer awareness , etc

Member Roles:-

I.. Saksham Jain (BS-MS 2019):-

Roles: **Team lead,** Human Practices, Entrepreneurship

Pre majors: Biology, Chemistry, Mathematics

Skills: *Communication* (winner-clarion,conduction of surveys, webinars & events as EBSB core member), *Outreach*(relevant contacts in & outside bhopal), *teaching* (for RAA & webinars as ex-igem volunteer), *presentation & entrepreneurial skills*(runner-up Curveball), *MATLAB, python, HTML*

Interests: Microbiology, Oncology, Synthetic biology, Biostats, Reading, blog writing

II. Harshul Raj Surana (BS-MS 2019):-

Roles:- **Modelling**, Human Practices, Entrepreneurship

Pre majors:- Biology, Mathematics, Data Science

Skills:- MATLAB, Python, writing & editing, Biostatistics, ML, economics in +2, Social media handling, HTML/CSS, attend weekly startup pitches.

Interests: Computational biology, Data Science & ML, coding, writing

III. Anurag Yadav(BS-MS 2019):-

Roles:- **Designing**
Pre majors:- Biology, Chemistry, EES
Skills:- Vector designing, poster making, social media handling, reading scientific docs
Interests:- microbiology, genetics, & biochemistry

IV. Ashley Suraj Hermon(BS-MS 2019):-

Roles:-**Wet lab,** Experiment design, Content creation, procedure
Pre majors:- Biology, Chemistry, EES
Skills:- article writing, scientific reading, critical analysis, designing, outreach, social media handling
Interests:- Cancer biology, gene therapy, endocrinology.

V. Aditi Chaudhari(BS-MS 2019):-

Roles:-**Wet lab**, experiment design, Content creation, procedure
Pre majors:- Biology, Chemistry, EES
Skills:- Scientific reading, article writing, Machine Learning, Python, Data science
Interests:- Synthetic biology, immunology, genetics.

Thank You

Team members:- Saksham Jain, Aditi Chaudhari, Ashley Suraj Hermon, Harshul Surana, Anurag Yadav

References:-

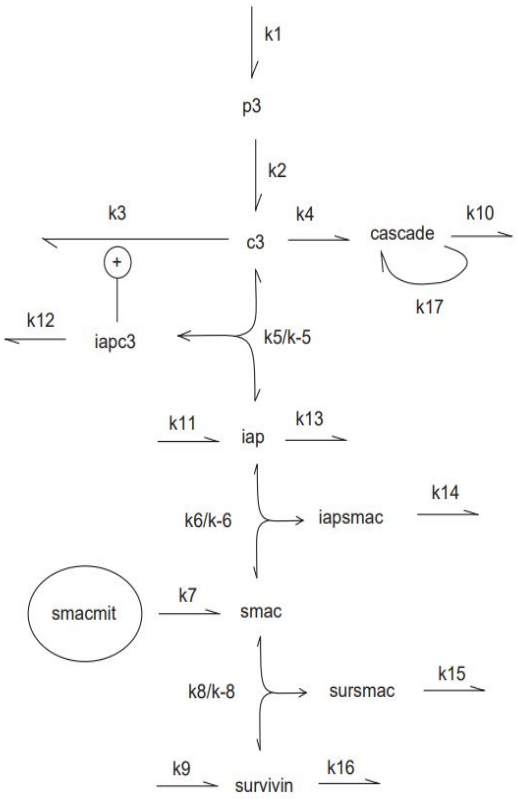
1)Xu, Y-F et al. “A New Expression Plasmid in Bifidobacterium Longum as a Delivery System of Endostatin for Cancer Gene Therapy.” *Nature News*, Nature Publishing Group, 27 Oct. 2006, www.nature.com/articles/7701003.

2)KM; Fulda S; Wick W; Weller M; Debatin. “Smac Agonists Sensitize for Apo2L/TRAIL- or Anticancer Drug-Induced Apoptosis and Induce Regression of Malignant Glioma in Vivo.” *Nature Medicine*, U.S. National Library of Medicine, www.pubmed.ncbi.nlm.nih.gov/12118245/.

3)Thornberry, NA., et al. “Smac/DIABLO Enhances the Therapeutic Potential of Chemotherapeutic Drugs and Irradiation, and Sensitizes TRAIL-Resistant Breast Cancer Cells.” *Molecular Cancer*, BioMed Central, 1 Jan. 1997, www.molecular-cancer.biomedcentral.com/articles/10.1186/1476-4598-7-60.

4)Ganai, S, et al. “Tumour-Targeted Delivery of TRAIL Using Salmonella Typhimurium Enhances Breast Cancer Survival in Mice.” *Nature News*, Nature Publishing Group, 27 Oct. 2009, www.nature.com/articles/6605403.

Modelling (elaboration):-



Name	Full form	Differential Equation
p3	Pro caspase 3	pc3dot = k1- k2[pc3]
c3	Caspase 3	c3dot = k2[pc3] - k3[lysis][c3] - k5[c3][iap] + km5[iapc3] - k10[c3]
iapc3	IAP–caspase-3 complex	iapdot = -k5[c3][iap]+km5[iapc3] -k6[iap][Smac] +km6[iapsmac] +k11 -k13[iap]
iap	inhibitor of apoptosis protein(XIAP here)	smacdot = k7[smacmit] -k6[smac][iap] +km6[iapsmac] - k8[smac][survivin] +k8[sursmac]
Smac	Smac	survivindot = k9 - k8[smac][survivin] +km8[sursmac] - k16[survivin]
iapsmac	IAP -Smac complex	iapsmacdot = k6[smac][iap] -km6[iapsmac] - k14[iapsmac]
smacmit	Smac in mitochondria	smacmitdot = -k7[smacmit]
surmacs	surviving–Smac complex	sursmacdot = k8[smac][survivin] -km8[sursmac] - k15[sursmac]
survivin	NA	iapc3dot = k5[c3][iap] -km5[iapc3] -k12[iapc3]

Material availability:-

Bacterium :- Bifidobacterium longum available at NCL repository, PUNE (cost ₹5000)

Genes available as bio-bricks:-

BBa_K1166004 - sTRAIL

BBa_K3419005 -Kill switch

BBa_K2348002 - m-Cardinal

BBa_K592101 - YFP

Plasmid pMB-1 available at many online websites (life-science market, creative biogene,etc.) (Cost approx $50)

Electroporator (if unavailable) - Electropen, a tool made by team Lambert iGEM 2018 (cost around $5)

Cell lines:- from Dr. Sanjeev shukla/ Dr. Sunando Dutta ‘s lab