Emerging Trends in Biosimilars and Biologics

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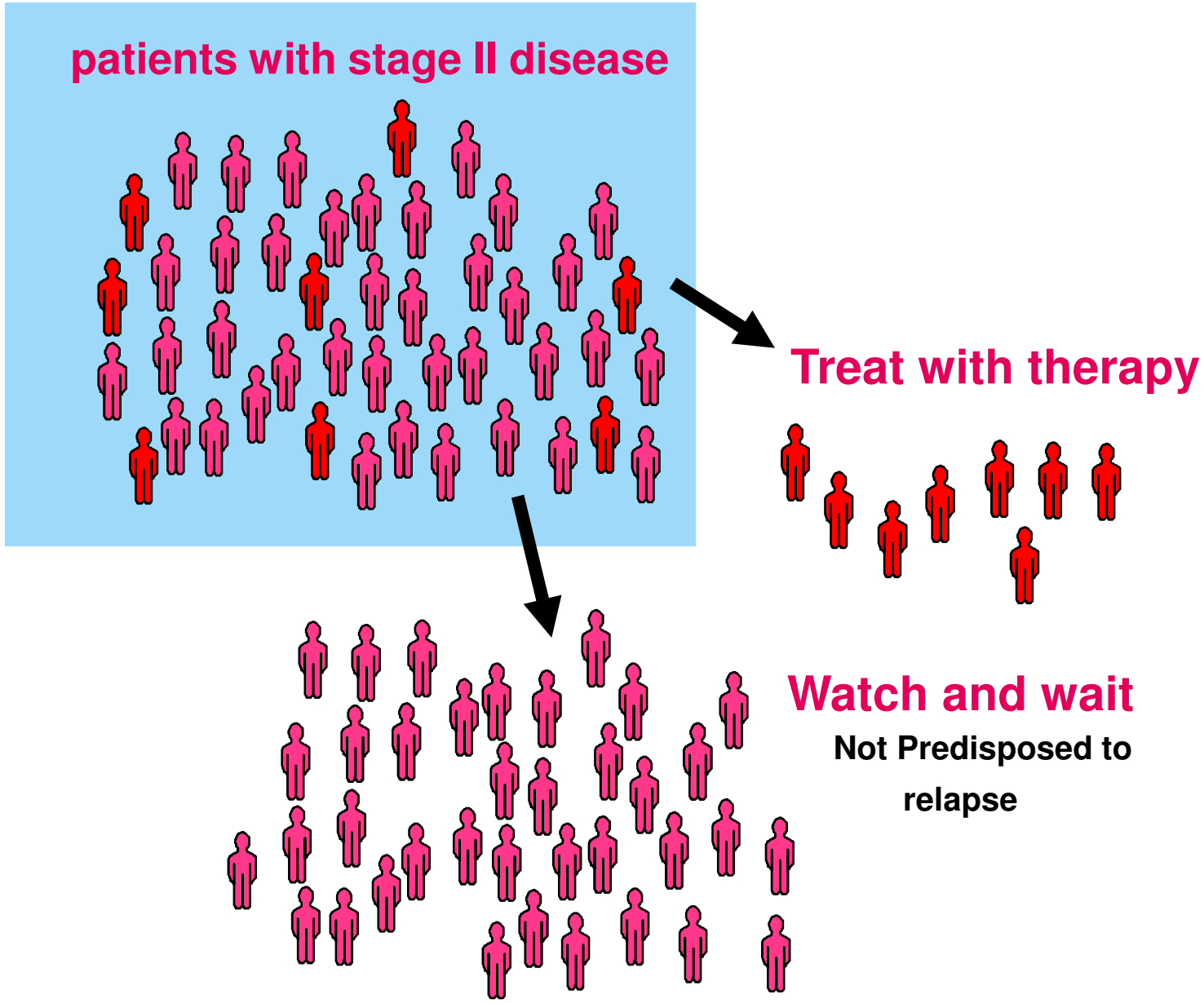
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Why Biologics and Biosimilars ?

- Acquired chemoresistance is often the cause of high mortality rate in cancer. Understanding its genetic mechanisms will enable us to design better biologics.
- G-protein coupled receptors (GPCRs) initiate multiple oncogenic signaling pathways in cancer cells by activating their associated G-proteins.
- We were successful in building a model of PAR2, being a family of GPCR; Activation of GPCRs by growth factors triggers survival signaling pathways that drive resistance to chemotherapeutic drugs such as cisplatin and taxane in female cancers. This is worrying situation.
- We found a number of molecules which could be suitable for some targets,
- These are listed in our publications.

Finding targets for B & B

- GPCR activation of G-proteins is opposed by the activity of regulator of G-protein signaling (RGS) proteins. RGS proteins inhibit G-protein signaling pathways by directly binding to the activated Gα subunit of G-proteins to accelerate hydrolysis of GTP into GDP, which returns G-proteins to an inactive state.
- Relevant to our studies, recent reports indicate that RGS proteins inhibit breast, lung, prostate, and ovarian cancer cell growth through inhibition of GPCRs signaling pathways.
- Hence these are important targets for biosimilars and biologics.


patients with stage II disease
Treat with therapy
Watch and wait
Not Predisposed to relapse

Her2/neu Receptor – approval

- Trastuzumab tested in breast cancer patients with HER2 overexpression and/or *HER2* amplification in their tumors.
- Detection of HER2 protein overexpression by immunohistochemistry (IHC) or *HER2* gene amplification by fluorescence in situ hybridization (FISH) was advised for selection of patients for trastuzumab therapy.
- Trastuzumab received FDA approval in 1998 for the treatment of HER2-overexpressing metastatic breast cancer, as a single agent or in combination with paclitaxel, in patients who have received one or more chemotherapy regimens.
- In 2006, trastuzumab was approved for adjuvant treatment of HER2-overexpressing breast cancer, either in combination with doxorubicin, cyclophosphamide, and paclitaxel or as a single agent following chemotherapy

Ras Proteins – Pre-Clinical

- Family of small GTPase protein which are involved in transmitting signals within cells (i.e., signal transduction)
- Ras is the most common oncogene in human cancer - mutations that permanently activate Ras are found in 20-25% of all human tumors and up to 90% in certain types of cancer (e.g. pancreas cancer).
- The three human *ras* genes encode H-Ras, K-Ras and N-Ras
- Overactive Ras signaling as a result of protein overexpression can ultimately lead to cancer.
- Ras protein myristylation (farnesylation, geranylgeranylation) is required for malignant transformation, these are some of the hot targets for biologics.

Identifying Biomarkers

- Epidermal growth factor receptor (EGFR) mutations as biomarker for head and neck squamous cell carcinomas (HNSCC). *K. Nagalakshmi**, Kaiser Jamil**, Usharani Pingali, M. V. V. Reddy, and Suresh S. V. Attili: 2014*. **Biomarkers**, Early Online: 1–9, 2014 Informa UK Ltd. DOI: 10.3109/1354750X.2014.895852
- Mutational Analysis of Erythropoietin Gene and Its Enhancer in Anemic Cancer Patients. *Kalyani P, **Kaiser Jamil**, Kirmani N, Nagalakshmi K, Mohan Reddy N, Archana.*

  Sch. J. App. Med. Sci., 2014; 2(3A):942-948.
- Induction of Apoptosis through Cox-2 and Bcl-2 activation by Gefitinib, Cisplatin and 5-FU in HeLa cells*. Musthaq Ahmed and **Kaiser Jamil*.* **(2013)** International **Journal of Biotechnology and Bioengineering Research**, 4(1): 47-62
- Genotypes of XRCC1 Polymorphism (Codon 399 Arg/Gln) and their Association with Lung Cancer. *Kirmani Natukula, **Kaiser Jamil** , Usha Rani Pingali, V.S. Suresh Attili, M.U. R. Naidu* **(2013); Asian Pacific Journal of Cancer Prevention**, 14(9): 5275-9.;

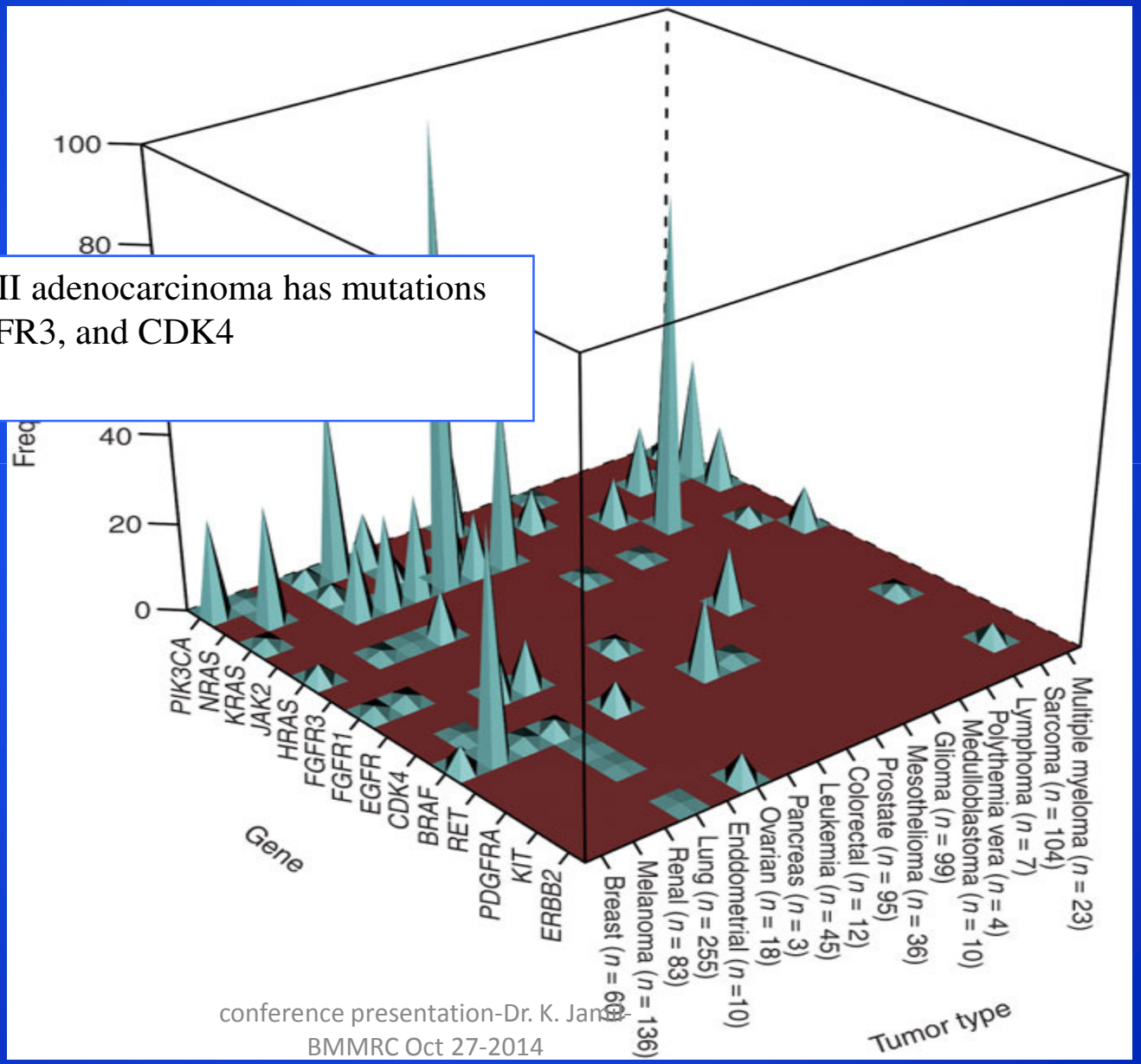
EGFR Receptor –

- Specific somatic mutations, small deletions, insertions, or point missense mutations in the EGFR tyrosine kinase correlate with better prognosis and increased objective response rate in NSCLC patients treated with small molecule TKIs but not with cetuximab
- *KRAS* mutations appear to predict for insensitivity of tumors to both antibodies and small molecules.

Mutations aplenty!

A patient with stage III adenocarcinoma has mutations in KRAS, BRAF, FGFR3, and CDK4

WHAT DO YOU DO?



Oncotype DX 21-gene recurrence score

16 cancer genes and 5 reference genes make up the Oncotype DX gene panel. The expression of these genes is used to calculate the recurrence score:

PROLIFERATION
Ki-67
STK15
Survivin
Cyclin B1
MYBL2

ESTROGEN
ER
PR
Bcl2
SCUBE2

BAG1

GSTM1

CD68

INVASION
Stromelysin 3
Cathepsin L2

HER2
GRB7
HER2

REFERENCE
Beta-actin
GAPDH
RPLPO
GUS
TFRC

RS =
+ 0.47 x HER2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

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BMMRC Oct 27-2014

Paik *et al. N Engl J Med.* 2004;351:2817-26.

Biomarkers potentially useful in cancer diagnosis

Biomarker	Cancer type	References
Apolipoprotein A1	Ovarian, pancreatic	Zhang et al., 2004; Kozak et al., 2005
Heptaglobin α-subunit	Ovarian, pancreatic, lung	Ye et al., 2003
Transthyretin fragment	Ovarian	Kozak et al., 2005
Inter-alpha-trypsin inhibitor fragment	Ovarian, pancreatic	Zhang et al., 2004
Vitamin D-binding protein	Prostate, breast	Corder et al., 1993; Pawlik et al., 2006
Serum amyloid A	Nasopharyngeal, pancreatic, ovarian	Orchekowski et al., 2005; Moshkovskii et al., 2005
α1-antitrypsin and α1-antichymotrypsin	Pancreatic	Orchekowski et al., 2005; Yu et al., 2005
Osteopontin	Ovarian, prostate	Khodavirdi et al., 2006

Response of genotypes to therapy

Table 20: Correlation of Genotypes with response of patients to chemotherapeutic drugs:

Genotypes & Response	MTHFR C677T	MTHFR A1298C	DPD IVS14+1G - A	TS 6bp/0bp	TS 2R/3R	CYP2D6 G1934A	SULT1A1 G638A
Wild Type							
Normal	16(43.24%)	17(37.77%)	47(60.25%)	48(58.53%)	63(81.81%)	41(48.80%)	46(57.5%)
Partial	21(56.75%)	25(55.55%)	31(39.74%)		14(17.07%)	31(36.90%)	29(36.25%)
Poor	0(0.00%)	3(6.66%)	0(0.00%)	28(34.14%)	5(6.09%)	12(14.28%)	2(2.5%)
				6(7.31%)			
Hetero							
Normal	3(9.3%)	4(17.39%)	0(0.00%)	4(14.28%)	3(20%)	0(0.00%)	3(15%)
Partial	9(28.12%)	9(39.13%)	2(25%)	13(46.42%)	4(26.66%)	8(57.14%)	10(50%)
Poor	19(59.37%)	12(52.17%)	4(100%)	11(39.28%)	8(53.33%)	6(42.85%)	1(100%)
Homo							
Normal	0(0.00%)	0(0.00%)	0(0.00%)	1(33.33%)	0(0.00%)	0(0.00%)	0(0.00%)
Partial	2(50%)	2(50%)	0(0.00%)	0(0.00%)	1(16.66%)	1(25%)	1(100%)
Poor	2(50%)	2(50%)	2(50%)	2(66.66%)	5(83.33%)	3(75%)	0(0.00%)

THE NEED FOR B & B

- In view of the heterogeneity of tumors, there is a need to develop new class of drugs which can distinguish cancer cells and from non-cancer cells.
- Most current oncology treatments seek to kill cancer cells directly whereas immuno-oncology drugs unleash the body's own ability to recognize and destroy cancer cells, which medical researchers say could have broader reach.
- We believe a combination of immuno-oncology agents represents the best chance for patients to achieve long-term survival.

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Structure and putative signaling mechanism of protease activated receptor (PAR2) – A promising target for breast cancer

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ABSTRACT

Experimental evidences have observed enhanced expression of protease activated receptor 2 (PAR2) in breast cancer consistently. However, it is not yet recognized as an important therapeutic target for

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RESEARCH ARTICLE

Screening of phytochemicals against protease activated receptor 1 (PAR1), a promising target for cancer

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Abstract

Context: Drug resistance and drug-associated toxicity are the primary causes for withdrawal of many drugs, although patient recovery is satisfactory in many instances. Interestingly, the use of phytochemicals in the treatment of cancer as an alternative to synthetic drugs comes with a host of advantages; minimum side effects, good human absorption and low toxicity to normal cells. Protease activated receptor 1 (PAR1) has been established as a promising target in many

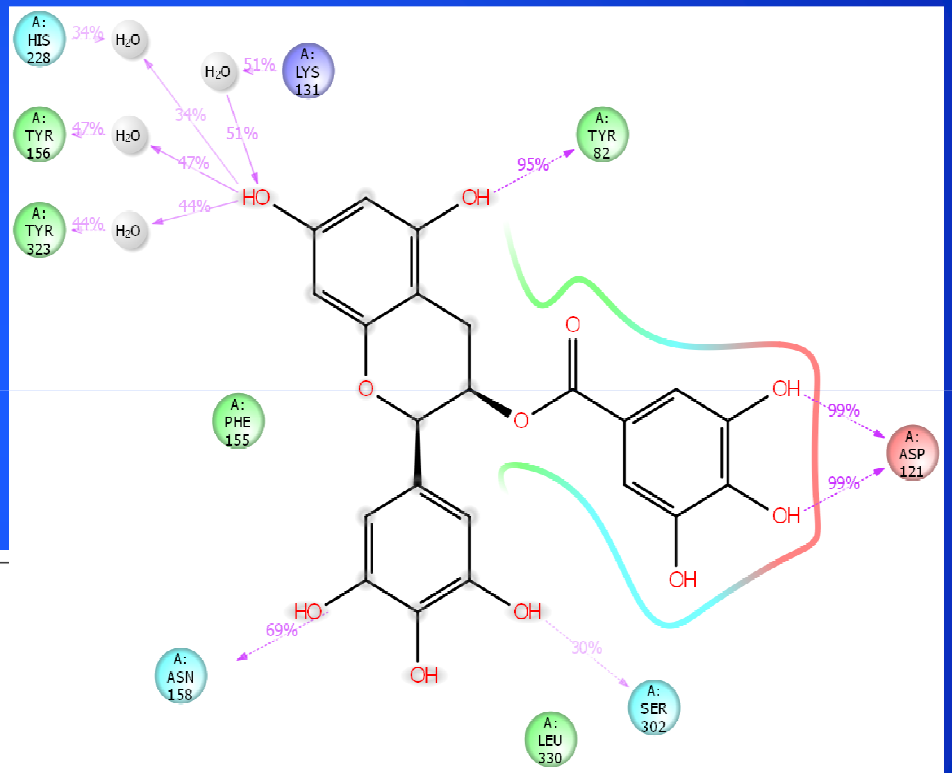
Keywords

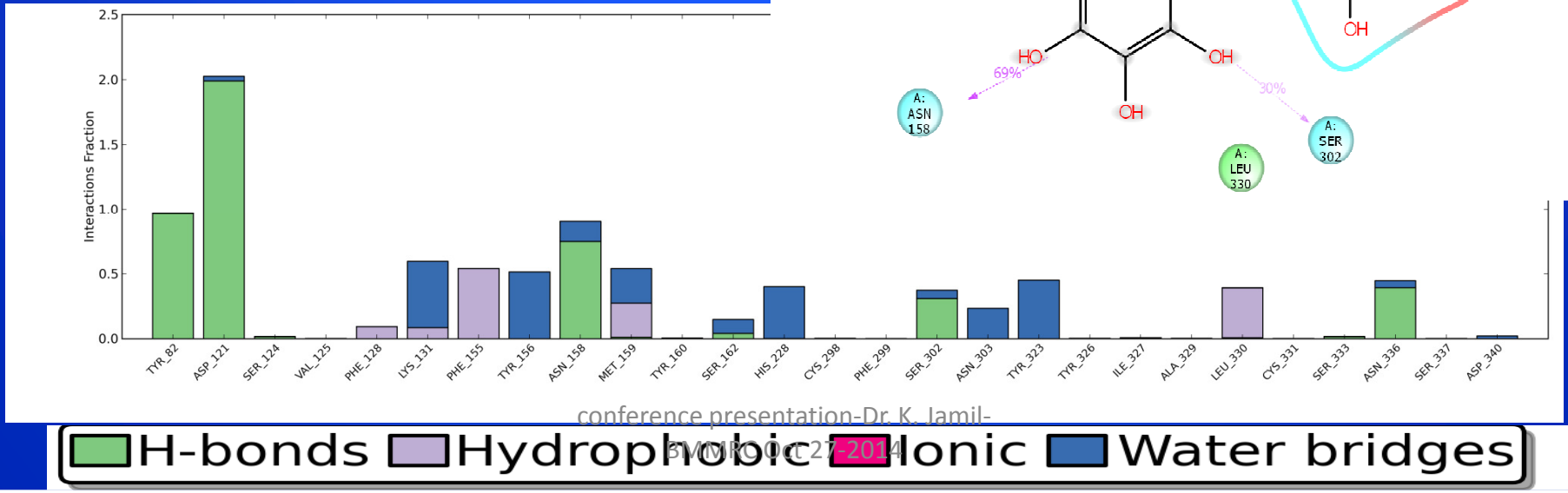
Docking, Glide, NPACT, phytochemicals, Protease activated receptor 1, Qikprop, virtual screening

Docking analysis and Molecular dynamics study of Protease activated receptor (PAR2) with Phytochemicals: Target for Breast cancer.

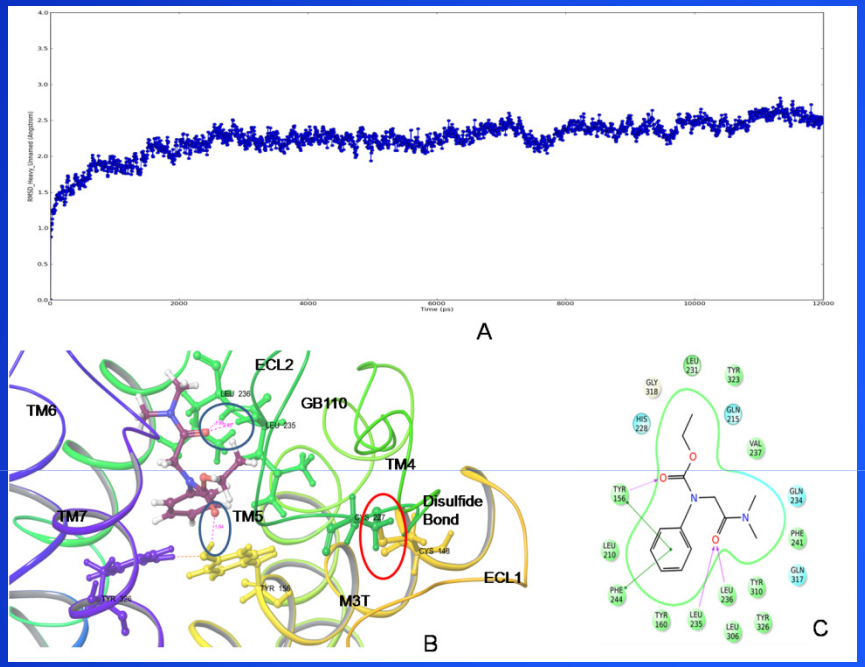
Table 3. Glide score, Induced fit docking score and MMDBSA values of Phytochemicals screened.

S.No	Pubchem ID & Entry Name	Glide g score	IFDScore	MMGBSA dG_bind (Kcal/mole)
1.	**CID_65064_Epigallocatechin-3-gallate (EGCG)**	**-19.457**	**-567.0666**	**-128.894**
2.	**CID_12306736_Mangiferin**	**-18.017**	**-560.6741**	**-107.393**
3.	**CID_5281544_Oleuropein**	**-15.855**	**-560.5267**	**-109.052**
4.	*CID_639665_Xanthohumol*	*-13.273*	*-560878*	*-113.294*
5.	CID_2889-curcumin	-12.857	-558.863	-112.826,
6.	CID_124052_Glabridin	-11.825	-557.728	-98.442
7.	CID_5318517_Andrographol			-111.249





3-D modeling of receptor domains of PAR2





Trajectory Cluster representatives	Glide score	IFD score	Prime/MMGBSA dG Bind (Kcal/mole)
Cluster 1	-7.679	-513.608	-82.462l
Cluster 2	-9.731	-551.585	-92.224
Cluster 3	-8.359	1163	-86.693
Cluster 4	-8.660	-108.687	-83.066
Cluster 5	-7.979	-538.179	-64.309

Computer Science
Systems Biology

Jayaraman et al., J Comput Sci Syst Biol 2013, 6:3
http://dx.doi.org/10.4172/jcsb.1000111

Research Article Open Access

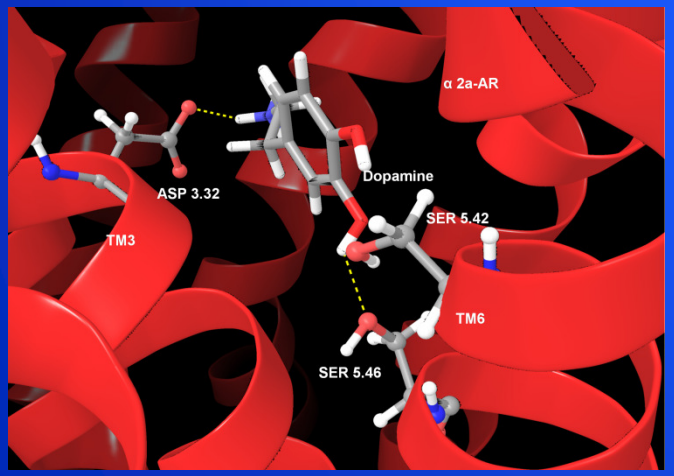
Homology Modelling and Docking Studies of Human α2-Adrenergic Receptor Subtypes

Archana Jayaraman, Kaiser Jamil and Kavita K Kakarala*

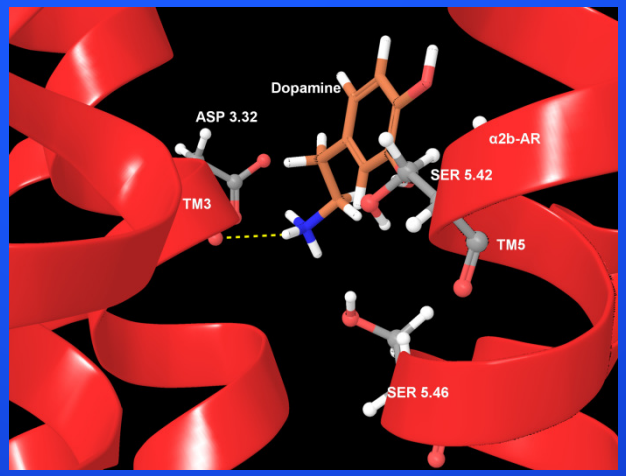
Centre for Biotechnology and Bioinformatics (CBB), School of Life Sciences, Jawaharlal Nehru Institute of Advanced Studies (JNIAS), Secunderabad, Andhra Pradesh, India

Abstract

α2-adrenergic receptors play a key role in the regulation of sympathetic system, neurotransmitter release, blood pressure and intraocular pressure. Although α2-adrenergic receptors mediate a number of physiological functions *in vivo* and have great therapeutic potential, the absence of crystal structure of α2-adrenergic receptor subtypes is a major hindrance in the drug design efforts. The therapeutic efficacy of the available drugs is not selective for subtype specificity (α2a, α2b and α2c) leading to unwanted side effects. We used Homology modelling and docking studies to understand and analyze the residues important for agonist and antagonist binding. We have also analyzed binding site volume, and the residue variations which may play important role in ligand binding. We have identified residues through our modelling and docking studies, which would be critical in giving subtype specificity and may help in the development of future subtype-selective drugs.



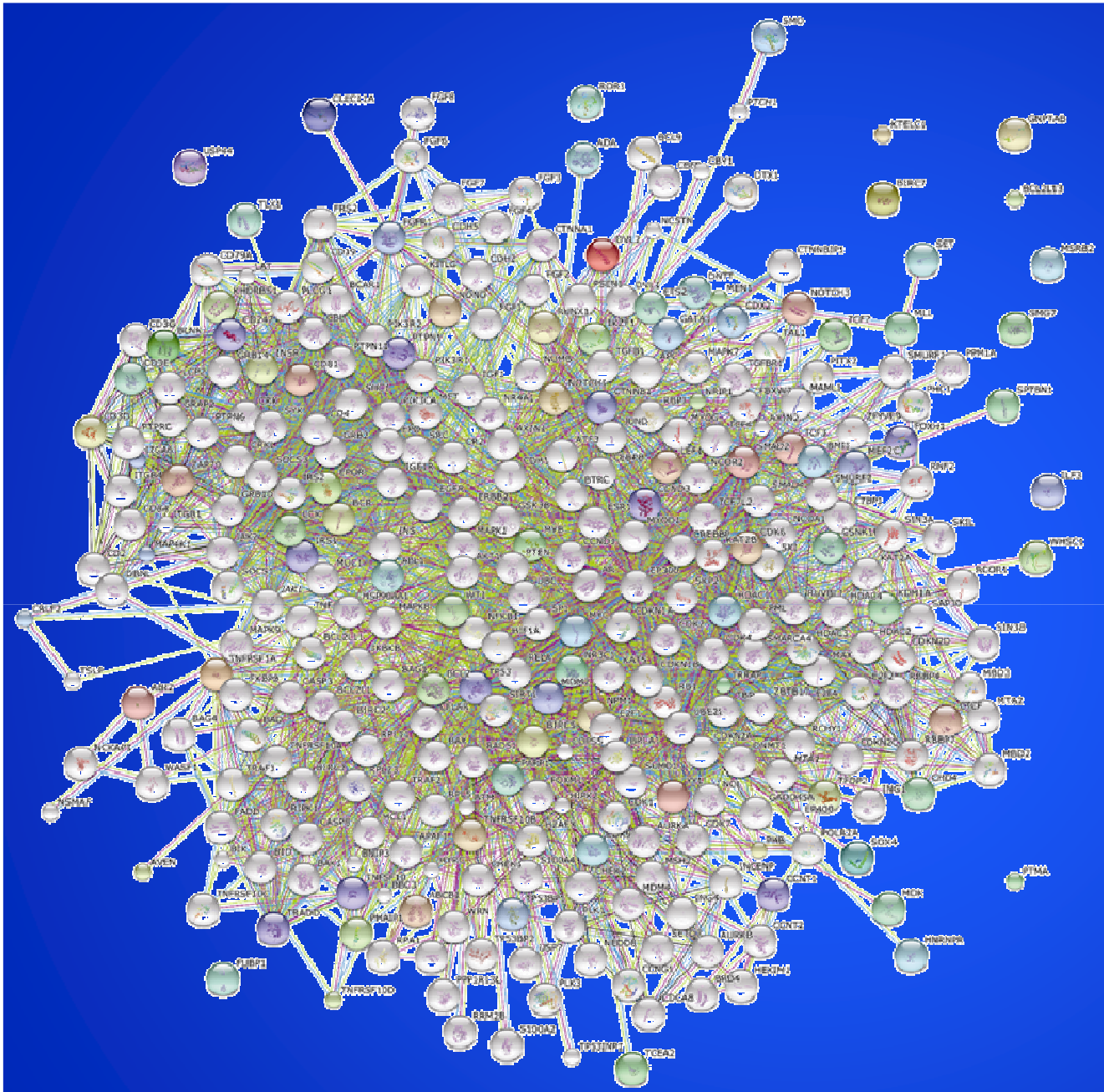
α 2a- adrenergic receptor



α 2b- adrenergic receptor

Models of alpha adrenergic receptor subtypes were validated by docking with known agonist (dopamine) before using them for further interpretations.

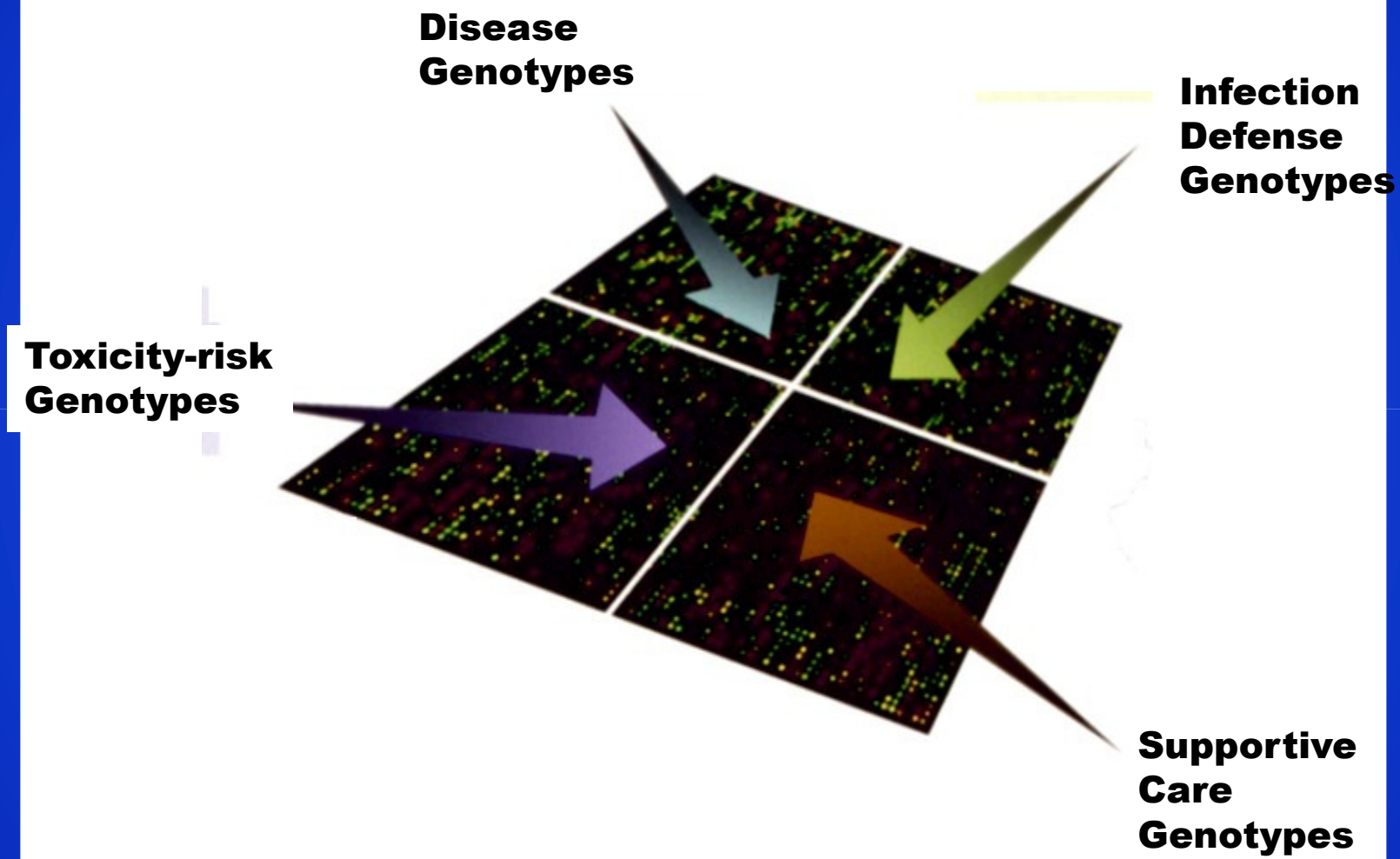
All the models reproduced experimental results confirming the suitability of models for further studies.



Protein-Protein Interaction network using Candidate and training proteins

- Network interactors' pathways –
- Cell cycle,
- apoptosis regulation, **p53,**
- T-cell and B-cell receptor, **MAPK, Wnt, ErbB, Notch, TGF-beta (TGF β)** signaling pathways,
- Focal adhesion, Hematopoietic cell lineage, cytokine-cytokine receptor interaction
- *High interconnectivity of these pathways-cooperative mechanisms of leukemic blast cell propagation*

Comprehensive optimization of patient care



- In a recent melanoma paper, Bernards et al. show that a decrease in SOX10 expression leads to increased EGFR expression and development of resistance to BRAF inhibitors.
- Removing BRAF or MEK inhibitors reduces melanoma cell proliferation and induces senescence in these cells.
- The authors suggest that a "drug holiday" may reverse EGFR expressionand resensitize melanoma cells to BRAF inhibitors.(Nature2014)

Developing a Personalized Medicine

- As the development of oncologics and other specialty and biologics become increasingly targeted, pharmaceutical manufacturers must develop new approaches to demonstrating value.
- Often times the standard approaches to the generation of cost-effectiveness and outcomes evidence do not adequately address particular aspects that the new medicine delivers to patients, payors, and society.
- In this presentation we will examine new approaches to unlocking value for targeted therapies including those with companion diagnostics.

New inhibitors

- **HIF Inhibitor [BAY 87-2243]** is a potent and selective hypoxia-inducible factor-1 **(HIF-1)** inhibitor with **IC50** of 0.7 nM and 2 nM.

  Related Products: FG-4592, 2-Methoxyestradiol, IOX2

- **Rho Inhibitor- K-Ras(G12C) inhibitor 9** is an allosteric inhibitor of oncogenic K-Ras(G12C).

  Related Products: EHop-016, K-Ras(G12C) inhibitor 6

- **ERK Inhibitor- GDC-0994** is a potent, orally available **ERK1/2** inhibitor with **IC50** of 1.1 nM and 0.3 nM, respectively. Phase 1.

  Related Products: XMD8-92, FR 180204, SCH772984

- **mTOR Inhibitor- Zotarolimus** (ABT-578) is an analogue of rapamycin, and inhibits **FKBP-12** binding with **IC50** of 2.8 nM.

  Related Products: Everolimus, Rapamycin, AZD8055

- **Cysteine Protease Inhibitor- PD 151746** is a selective, cell-permeable **calpain** inhibitor with **K_i** of 0.26 M for μ-Calpain, about 20-fold selectivity over m-calpain.

  Related Products: E-64, Aloxistatin, Loxistatin Acid

SUGGESTIONS FOR DEVELOPMENT OF B & B PRODUCTS:

- PROTEIN ENGINEERING & DEVELOPMENT
  - Recombinant Protein Therapeutics
  - Enhancing Antibody Binding and Specificity
  - Improving the Clinical Efficacy of Antibody Therapeutics

- ANTIBODY THERAPEUTICS
  - Cancer Targets for Antibody Therapeutics
  - Antibody-Drug Conjugates
  - Bispecific Antibody Therapeutics

- - Engineering Genes, Vectors, Constructs and Clones
  - Recombinant Protein Expression and Production
  - Transient Protein Production

Targets

- Complicated networking of proteins!
- **Kaiser Jamil (2012).** Cancer communications for the development of personalized medicine.[Editorial]. **Journal of Solid Tumors, (Canada),** 2(2): 1-3. (43 Downloads)

- **Kaiser Jamil** and Sabeena Mohammed Mustafa (**2012**). Thioredoxin System: A Model for Determining Novel Lead Molecules for Breast Cancer Chemotherapy. **Avicenna Journal of Medical Biotechnology**, 4(3): 1-10. [PMID: 23407461]
- Mushtaq Ahmed,D. Jayasimha Rayalu, **Kaiser Jamil** (**2012).** Molecular docking studies targeting cyclooxygenase-2 (COX2) involved in cancer. **International journal of Pharmaceutical Sciences and Healthcare,** 4(2): 76-85. ISSN 2249 -5738.

Epigenetics

- Epigenetic changes in different classes of this type of cancer have been studied, including: estrogen receptor positive (ER+), that are estrogen-level dependent; estrogen receptor negative (ER-), whose tumor cells are not responsive to estrogen thus resistant to antiestrogenic drugs such as tamoxifen and aromatase inhibitors; progesterone receptor (PR); and human epidermal growth factor 2 (HER2)-related cancers .

- A number of genes has been identified to be aberrantly methylated in breast cancer and their number is rapidly growing.

- . Likewise, altered expression of micro RNAs has been found to regulate key genes in the development of breast cancer . Biological rationales for breast cancer therapies have been deeply studied by inhibiting DNA methyltransferases (DNMT) and histone deacetylases (HDAC) proteins.
- Furthermore, several epigenetic-based synthetic drugs, which can reduce DNA hypermethylation and histone deacetylation, are undergoing preclinical and clinical trials

- These *epidrugs* are a promising strategy for breast cancer therapies as they could restore the estrogen receptor α (ERα) activity in ER- cancer patients, reactivating cancer cell growth in an estrogen-dependent manner resulting sensible to antiestrogenic drugs

Cipla --for Avastin, Herceptin and Enbrel

- **Humanized Antibodies**
- The more successful biologics in the market are Herceptin for Breast cancer and bevacizumab and cetuximab for lung cancer.
- In general Biosimilars and biologics have proven useful in the treatment of hematologic malignancies like leukemia and lymphoma and they are being developed against solid tumors.
- All promising Biosimilars and biologics are in various phases of clinical trials and others in the pipeline, but we first need to understand it mechanism of action and its suitability as monotherapy, since in oncology, combinations typically provide superior benefit as we know that cancer is not a single disease.

Increase of Similar Biologics in India

- Biologics are an important component of the pharmaceutical industry and have grown exponentially in the last decade. In recent years, the pharmaceutical industry has placed greater and greater emphasis on developing biopharmaceutical-based drugs (biologics). As a result, the global biologics market is expected to reach $220 billion by 2019.
- In 2012, CDSCO, in collaboration with the DBT, issued the *Guidelines on Similar Biologics: Regulatory Requirements for Marketing Authorization in India* . The Guidelines detail the regulatory requirements, such as data requirements for the manufacturing, characterization, preclinical studies and clinical trials, for receiving marketing authorization of similar biologics. The Guidelines are applicable for similar biologics developed in or imported into India.
- The regulatory bodies responsible for approval of 'similar biologics' in India are the Department of Biotechnology (DBT – under the Ministry of Science and Technology), through its Review Committee on Genetic Manipulation (RCGM), and the Central Drugs Standard Control Organization (CDSCO – under the Ministry of Health and Family Welfare).

Biosimilars

- Several different BoNTA products are marketed in various countries, and they are not interchangeable. Differences between products include manufacturing processes, formulations, and the assay methods used to determine units of biological activity. These differences result in a specific set of interactions between each BoNTA product and the tissue injected.
- Botulinum toxin type A (BoNTA) products are injectable biologic medications derived from *Clostridium botulinum* bacteria.

Towards personalizes medicine

- In this genomics age, we know that diseases are partly the result of how genes interact with environmental and behavioral risk factors, such as diet and physical activity, hence we must begin to link genomic discoveries to appropriate population level assessments, policies and disease prevention programs.
- Eventually genomics will help to change the face of public health by focusing interventions on individuals and groups who will benefit the most from behavioral modifications, drug therapies, and other forms of interventions.

- Although the clinical trial with temsirolimus, an mTOR inhibitor, did not show any benefit when compared with endocrine therapy alone, a Phase II clinical trial with sirolimus has been reported to be promising.
- Recently, everolimus was approved in combination with exemestane by the US Food and Drug Administration for treating postmenopausal women with advanced HR+ breast cancer, based on the results of a Phase III trial.
- Therefore, everolimus represents the first and only targeted agent approved for combating endocrine resistance.

Conclusion/ Economics

- There is a growing market for Biosimilars and Biologics,
- We learn that Biopharma companies are competing for these bioproducts:
- Vantictumab combined with a standard chemo therapy are now in three Phase Ib trials in non-small cell lung cancer, HER2-negative breast cancer and pancreatic cancer.
- (Bayers)The pharma company signed up for a $430 million partnership in 2010, paying $40 million upfront for the right to buy in to up to 5 drug candidates.
- U.S. regulators are likely to approve Merck & Co's highly anticipated immuno-oncology drug, pembrolizumab, as a treatment for melanoma well ahead of a deadline, according to three sources familiar with the situation.(Reuters).
- If approved by the Food and Drug Administration, the drug would be the first in a promising new class designed to help the body's own immune system fend off cancer by blocking a protein known as Programmed Death receptor (PD-1), or a related target known as PD-L1, used by tumors to evade disease-fighting cells.

- Companies including Bristol-Myers Squibb, Roche Holding AG and AstraZeneca Plc are racing to develop similar treatments for a variety of cancers. Some analysts expect the new class could generate more than $30 billion in annual sales worldwide by 2025.
- Roche, which has a full pipeline of ADC projects, recently outlined plans to invest more than $200 million to build a new ADC facility in Basel. And *Chemical & Engineering News* notes that Sigma-Aldrich, Carbogen Amcis, Lonza and Piramal Healthcare have all recently announced new investments in ADC production facilities.
- Most current oncology treatments seek to kill cancer cells directly whereas immuno-oncology drugs unleash the body's own ability to recognize and destroy cancer cells,


Thank
you!