Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 21 Issue 3 Version 1.0 Year 2021
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

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*GJMR-B Classification:* *NLMC Code: QV 704*


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# Effect of Monoclonal Antibodies Conjugation with Gallium-Containing Solamargine: Warburg Effect-Based Cancer Therapeutic Strategy. Article Review

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## I. Introduction

Monoclonal antibodies constitute hoping results for malignancy treatment. Cancer cells have the ability to invade the immune system to predispose invasion and metastasis. Monoclonal antibodies target specific antigens presenting on cancer cells, in a result more specificity for cancer treatment with less side effects. The main role of them is to suppress main checkpoints that are critical for malignancy dissemination such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and tyrosine kinase. Many examples of monoclonal antibodies are produced against specific cancer cell antigens such as antibodies that protects immune checkpoints from cancer cell aggression like lipilimumap, pembrolizumab and alemtuzumab. Also Bevacizumab (anti–VEGF) inhibits the angiogenic activity of VEGF expressing cancer cells. Bevacizumab showed satisfying results in the treatment of metastatic colon cancer, metastatic renal tumors besides non-small cell lung cancer and glioblastoma. Panitumumab (anti-EGFR) is responsible for treatment of metastatic colon cancer expressing epidermal growth factor receptor (EGFR) which has been shown resistance to chemotherapeutic agents. Cetuximab (anti-EGFR) inhibits EGFR related pathways and is used in the treatment of EGFR-positive colon malignant neoplasm and also for head and neck tumors. Ofatumumab showed high efficacy in the treatment chemo-resistant patients with chronic lymphocytic leukemia chemotherapy. Trastuzumab (anti- HER-2/neu) is used in patients with HER-2/neu-positive breast tumor, metastatic gastrointestinal (GI) malignant neoplasms *[Pento, 2017]*. Rituximab has been a cornerstone in treatment of non-Hodgkin's lymphoma, lymphocytic leukemia and other autoimmune diseases such as lupus erythematosus *[Smolej, 2016]*. Monoclonal antibodies have been recognized for radioisotopes delivery such as arcitumomab that is a murine antibody fragment that is technetium 99m- labeled. It is a therapeutic agent for patients with metastatic colorectal neoplasm *[Hughes et al, 1997]*. Ibritumomab tiuxetan *(fig. 1)* can be tagged with yttrium 90 or Indium 111 which showed high efficacy in treatment of patients with non-Hodgkin's lymphoma and regularity combined with Rituximab *[Rizzieri, 2016]*. Tositumomab *(fig.2)* (a MAB labeled) is labeled with iodine 131 used for treatment patients with non-Hodgkin's lymphoma who show bad outcome to other chemotherapeutic drugs *[Shadman et al, 2016]*. Also monoclonal antibodies can be labeled by chemotherapeutic agent [chemolabeled antibodies-brentuximab vedotin (Adcetris)]. Another group of monoclonal antibodies are available which is called bispecific mAbs, that has double variable antigen binding fragments (Fabs) whose advantage is to attract cells together. For example, blinatumomab binds CD19 on lymphoma cells and CD3 on T cells, thus prompting T cell cytotoxicity against leukemic B cells *[Goldenberg, 2007]*.

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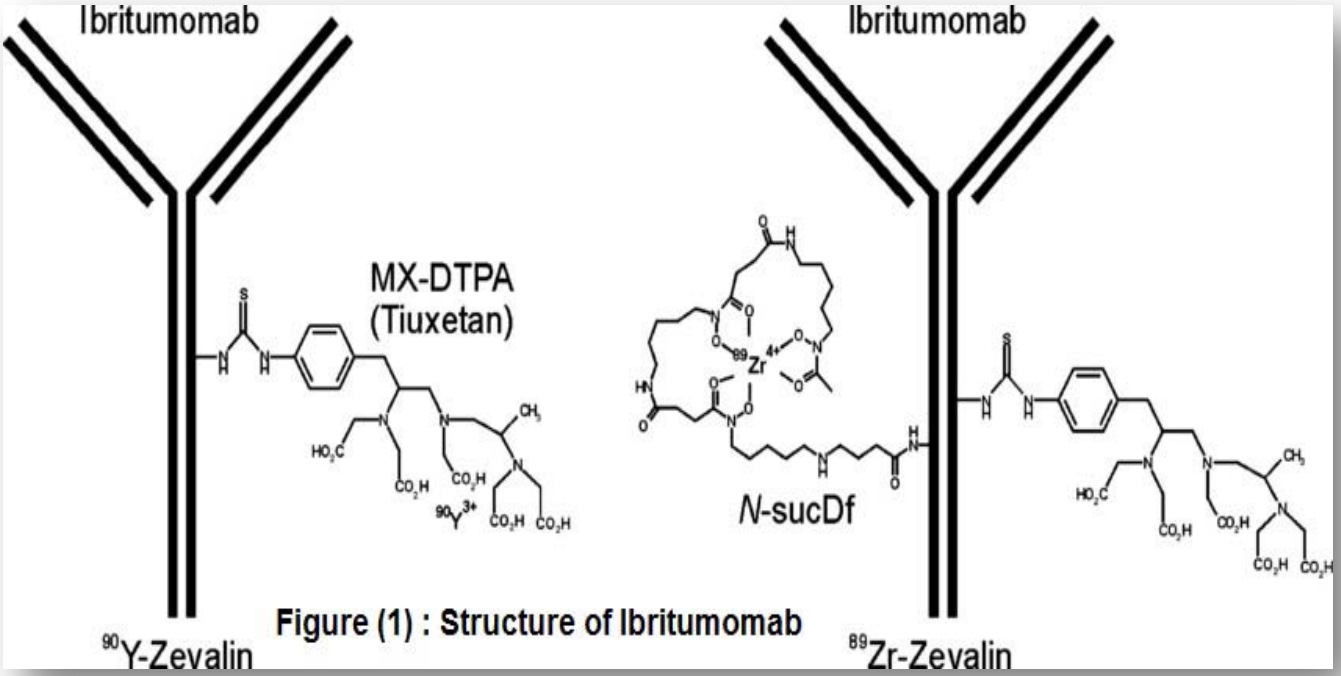



*Figure (1):* Ibritumomab tiuxetan structure: Tagging Fc portion with yttrium 90 or Indium 111.

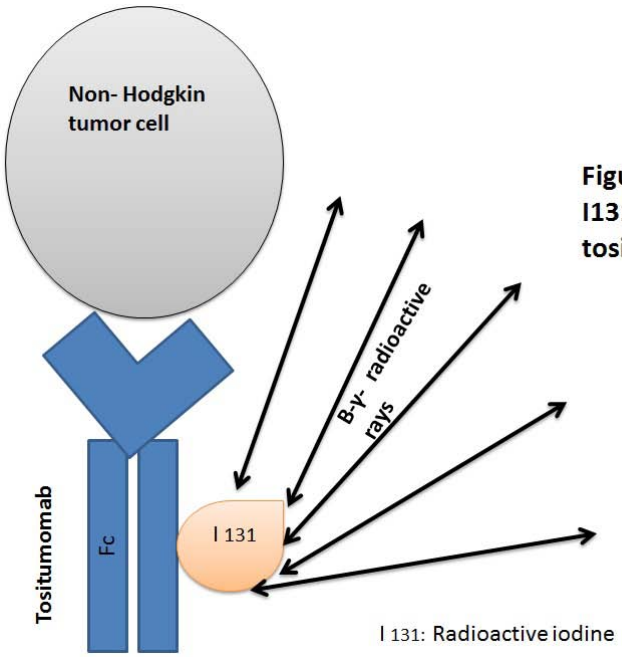



**Figure (2) : Radioactive iodine I131 attached to Fc region tositumomab**

*Figure (2):* Tositumomab labeling with iodine 131 used for treatment patients with non-Hodgkin's lymphoma by emission β, γ rays.

## II. Structural Insights of Monoclonal Antibody

The general conformation of monoclonal antibody consists of three functional components, two Fragment antigen binding domains (Fabs) and the fragment crystallizable (Fc), with a hinge region between the two Fabs and the Fc that gives the advantage of wide range of flexible mobility to the Fabs. Each of the Fabs contain identical antigen-binding sites that bind with a specific antigen *[Chiu et al, 2019].* The antigen binding sites of antibodies often results in structural variations in the contact surface zones of both the antibody and the antigen. That have been confirmed in the structure studies of both an antibody fragment (Fabs or Fvs) alone and bounded form with its antigen *[Davies*

*and Cohen, 1996].* The Fv region of the Fab consists of a pair of variable domains (VH and VL) together with the HC and LC. In contrast, the glycosylated Fc region binds to variable structures presented on malignant cells and components of the adaptive and humoral immunity. Fc region structure is nearly constant in many human IgG antibodies. It is formed of two constant domains, each one consists of CH2 and CH3. CH3 of both domains are joined tightly together, while CH2s have no protein–protein communication with each other *(fig.3).* The space in-between the CH2s is occupied partially by the carbohydrate attached at Asn297.In some antibodies, the two carbohydrate chains interact through hydrogen bonds or water bringing molecules. The flexibility of the CH2s has its role in the Fc region *[Chiu et al, 2019].* Interaction with Fc gamma receptors (FcR) and the first subcomponent of the C1 complex (C1q) to initiate antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP), trogocytosis, stimulation of mediators secretion, and endocytosis of opsonized particles *[Taylor and Lindorfer, 2015].* Now, The Fc region is the target of the developmental engineering for variable effector functions. Structural analysis showed a Fc ball-and-socket joint between CH2 and CH3 that permits the CH2 domain to circulate around its Leu251 side chain, which is buried in a pocket constituted of CH3 residues Met428, His429, Glu430, and His435. FcCH2 contains carbohydrate structures that conceal hydrophobic face of Fc region *[Chiu et al, 2019].* Several Fc glycoform variants and aglycosylated forms have been confirmed such as sialic acids, N-acetylglucosamines, and galactoses, and in some cases, the absence of fucose *[Jefferis, 2005].* Fc glycans improve the antibody biophysical stability *[Lee et al, 2015].* Also they fills the separation distances between CH2. Besides all that they can redirect the effector functionality of the antibody besides changing its the pharmacokinetic profile *[Kronimus et al, 2019].*

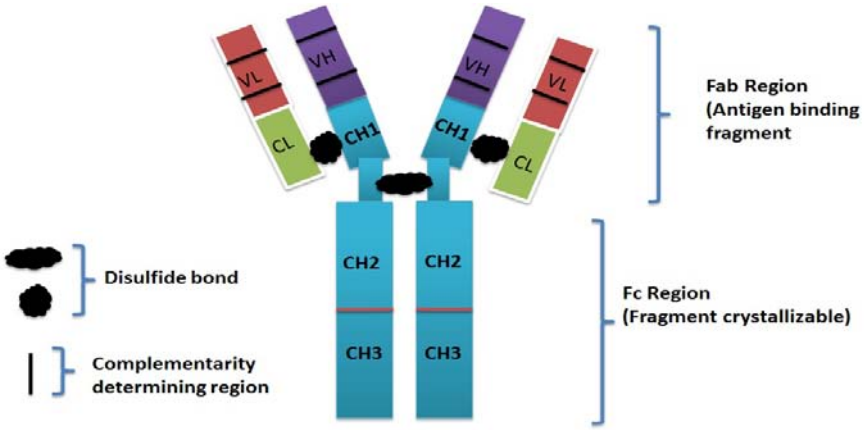



*Figure (3):* Monoclonal antibody consists of Fab region that contains antigen- binding domain (two variable heavy chains and two variable light chains). Fc region consists of CH2 and CH3. CH2 domain is the glycosylated.

a) *Genetic polymorphism and its effect on monoclonal antibodies efficacy*

Cancer cells have multiple genetic variations that affect monoclonal antibodies efficacy. Genetic polymorphism targets recognition, presentation and metabolism of monoclonal antibodies. Monoclonal antibodies maximum absorption 1-8 days after SC or IM injection *[Korth-Bradley et al, 2000]* and it is determined by blood-tissue hydrostatic gradient besides diffusion through vascular endothelium *[Baxter et al, 1994].* MABs uptake occurs after receptor-mediated endocytosis after binding of Fc domain with FcγR expressed on different immune cells *[Gessner et al, 1998].* However in a recent study, it has been shown that immune system has a necessary role in survival tumor cells that show loss of tumor suppressor genes or activated oncogenes *[Timothy et al, 2021].* Besides that, MABs can bind neonatal Fc receptor (FcRn) at its Fc region (CH2-CH3) domain interface (Ile 253 and two central histidines His 310 and His 435. FcRn protects MAB from intracellular degradation through intracellular recycling mechanisms *[Pyzik et al, 2019].* Reduced expression of FcRn by genetic mutation leads to decreased serum level of MAB and increased clearance ratio *[Ryman and Meibohm, 2017].*

Genetic mutations of cancer cells have inhibitory results on MAB efficacy. For example, BRAFV*600*E, PI3K/ m TOR and PI3K CA genetic mutations expressing colorectal cancer cell lines are associated with low cetuximab and panitumumab efficacy in colorectal cancer treatment potency *[Xu et al, 2017].* Patients that show RAS gene KRAS G*12* A/V

mutation that upregulates VEGF show lower PFS and OS after treatment with bevacizumab (anti-VEGF) compared to wild type KRAS *[Nakayama et al, 2017]*.

SNPs (single nucleotide polymorphisms) within the PD-L1 gene CD274 have been demonstrated to affect patient improvement to the anti-PD-1 mAb nivolumab. Patients with non-small cell lung cancer that administrated nivolumab possessing the CD274 rs4143815 C/C and C/G genotypes had slightly more elevated median PFS in comparison to patients with the G/G genotype (P = 0.044). Also several studies suggested that PD-L1 rs4143815, that is situated in the 3' untranslated region (UTR) can affect the expression of PD-L1, in a result tumor cells can escape from immune system *[Yeo et 2017]*. Especially, it has been proven that the C allele of rs4143815 has an essential role in an increased production of PD-L1 by attenuating miR-570 *[Wang et al, 2013]*. Also it is clear that patients with the rs4143815 C/C genotype have lower clinical result to paclitaxel and cisplatin chemotherapy *[Lee et al, 2016]*. In addition, during the haplotype analysis, that included seven SNPs (rs733618, rs4553808, rs11571317, rs5742909, rs231775, rs3087243 and rs7565213) within CTLA4 gene, it can be associated with no response to anti-CTLA-4 treatment *[Breunis et al, 2008]*.

*b) Value of monoclonal antibodies conjugation with solamargine-gallium containing saccharide*

i. *Value of solamargine*

1. Glycoside nature of solamargine: tumor cells have increased needs of glucose for their high rate of replication and invasion. Malignant cells prefer aerobic glycolysis other than mitochondrial oxidation. Rate of glucose metabolism by aerobic glycosylation is roughly 10 -100 more rapid than that of mitochondrial oxidation *[Locasale and Cantley, 2011]*. Also aerobic glycolysis results in production considerable amount of lactic acid after glucose fermentation in the presence of oxygen and functioning mitochondria which is called "Warburg effect". Lactic acid is important for tumor survival and progression *[Maria et al, 2015]*. Besides that, aerobic glycolysis satisfy cancer cell needs of the high requirement of ATP which is necessary for tumor cells division *[Epstein, et al. 2014]*. Also, aerobic glycolysis is considered major factor for carbon production that is crucial for formation of nucleotides, lipid and protein for cancer anabolism and carcinogenic- associated pathways *[Boroughs and DeBerardinis, 2015]*. Warburg effect is essential for NAD+ regeneration that is important for keeping glycolysis active *[Lunt and Vander Heiden, 2011]*. In tumor microenvironment, glucose supply is limited. So tumor cells, stromal cells and immune cells compete for glucose consumption *[Chang C-H, et al, 2015]*. Also within the tumor, glucose as a nutrient is needed for tumor- infiltrating lymphocytes (TILs) for their effect or functions, also it is needed for cancer cell itself. Warburg effect through high aerobic glycolysis within tumor cells compensate TILs for glucose needs *[Chang C-H, et al, 2015]*. Warburg effect has unforgettable role in oncogene mutations such as KRAS in pancreatic cancer and BRAF in melanoma *[Shain AH, et al, 2015]*. From all previous advantages of raid aerobic glycolysis and Warburg effect, we can say that tagging monoclonal antibodies glycosides Fc region with solamargine (glycoside which has high affinity for cancer cells) can be attracting factor for malignant as it can be a glucose supply for their Warburg effect, especially within the low glucose nutrient in the tumor microenvironment (author).
2. Solamargine anti-cancer properties: solamargine is considered one of the glycoalkaloid class (solasodine rhamnosyl glycosides) which shows positive immune response to cancer cells. Solasodine rhamnosyl glycosides are secondary metabolites of plants. They consist of a mono or oligosaccharide chain attached at the C3 position of the nitrogenous steroid alkaloid backbone *[Bill, 2013]*.

Solamargine (SM) molecular formula is C45H73NO15 with the mass of 868.04 Da. Its systematic name is (22R, 25R)- spiro-5-ene-3ß-yl-α-L-rhamnopyranosyl-(1-2glu)-0-α-L-rhamnopyranosyl-(1-4glu)-ß-D-glucopyranose *(fig.4)*. It is chosen from solasodine glycosides because it contains D-glucopyranose which can be conjugated with gallium particles (discussed later.) Solamargine uptake by endogenous endocytic lectins (EELs) expressed on malignant cells results in cellular shrinkage and lysis *[Bill, 2013]*.

The 2 rhamnose moiety of SM have a necessary function in initiation cell death by apoptosis and cytotoxic effects such as human hepatocytes (Hep3B) *[Nakamura et al, 1996]*. It was observed that the carbohydrate moieties of steroidal alkaloids augmented the binding specificity to steroid-associated receptors *[Chang et al, 1998]*. The trisaccharide of SM (two rhamnose units are bound to a glucose moiety), has more affinity to specific cell receptor sites than the corresponding trisaccharide of solasonine (SS) (one rhamnose and one glucose units are connected by a galactose monosaccharide) *[Bill, 2013]*.

Solamargine has potent multiple anti-tumor properties. It showed efficient results in MDR (multiple drug resistance) tumor cells. Solamargine has shown high potency by apoptosis induction in Ehrlich Carcinoma, Leukemia (K562), Colon Cancer (HT-29, HCT-15), Liver Cancer (HepG2, PLC/PRF/5, SMMC-7721), Lung Cancer (A549), Gastric Carcinoma (AGS), Pancreatic Carcinoma (MIA, PaCa-2), Renal Adenocarcinoma (786-0) Uterine Adenocarcinoma

(HeLa 229), Ovarian Carcinoma (JAM), Mesothelioma (NO36),Glioblastoma, Astrocytoma (U87-MG), Prostate Carcinoma (DV-145, LNCap, PC-3), Melanoma (A2058), Breast Cancer (T47D, MDA-MB-231), Osteosarcoma (U20S) and Squamous Cell Carcinoma (A431, SCC4, SCC9, SCC25). Solamargine also showed selectivity as it did not induce apoptosis in normal cells such as bone marrow cells, fibroblasts, normal hepatocyte cells HL7702 and H9C2 *[Bill, 2013].*

The gene expression of TNFR1 was markedly increased by SM which contributes to the mechanism of the cytotoxicity of SM *[Hsu et al, 1996].* Solamargine triggers the intrinsic and extrinsic pathway of apoptosis in lung and breast cancer cells. SM increased the expressions of external death receptors, such as tumor necrosis factor receptor 1 (TNFR-1), Fas receptor, TNFR-1-associated death domain (TRADD) and Fas-associated death domain (FADD). SM also upregulated the intrinsic ratio of Bax to Bcl-2 by increasing Bax and reduction Bcl-2 and Bcl-xl expressions. As consequence, mitochondrial cytochrome c was released and activation of Caspase-8, -9 and -3 *[Bill, 2013].* Also, SM increased Fas expression and reduced the level of expressed HER2 receptor leading to increased sensitivity of trastuzumab and ant chemotherapy- induced apoptosis in NSCLC A549 and H441 cells, besides breast cancer cells *[Shiu et al, 2009].* Oncosis occurred at high doses of SM in the form of considerable conformations in cell shape and volume, blebs appearance on the cell membranes, mitochondrial swelling, clamping the nuclear contents and finally cell death. Apotosis occurs by low concentrations of SM and both types of cell death (oncosis and apoptosis) were resulted by intermediate concentrations of SM *[Sun et al, 2010].*

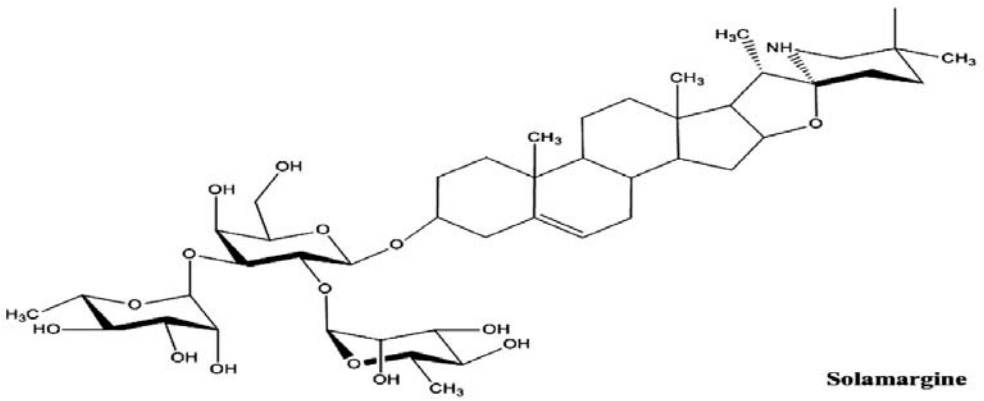



*Figure (4):* Structure of solamargine

3. Advantages of glycosylated antibodies: The main conserved glycosylation site of IgG is within the Fc region (Asn 297). Types of glycosides attached to Fc region of the monoclonal body determine the efficacy of monoclonal antibody. Antibody-dependent cell cytotoxicity (ADC in no- or low fucosylated MAB is stronger than highly fucosylated MAB. ADCC assay showed that monosialylation of IgG1 gave the advantage of Anti- D Mab for more cell lysis *[Kumpel et al, 1994].* Oligomannose intermediate terminal glycosylation decreased the potency of MAB four-six folds, while the exposed oligomannose structure fastens the monoclonal antibody clearance and reduces its concentration with the patient serum by binding with the mannose receptor on macrophages and phagocytic cells. Degree of galactosylation affects the strength of MAB related ADCC proportionally. In other words, hyper-galactosylation is considered a stimulator for FcγRIIIA– mediated ADCC but does not change the ability of MAB for constitution rosettes with cells that express high-affinity activating FcγRI (associated with myeloid cells) *[Kumpel et al, 1994].* In the opposite, hypogalactosylation results in weak activity of IgG in ADCC. IVIG (intravenous immunoglobulins) attained their efficacy by binding of it Fc region with FcγR-bearing host immune cells *[Galeotti et al, 2009].* That effect can be because of initiation secondary cellular events, like FcγR-induced apoptosis or anergy, including the phosphorylation of immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based activation motif (ITAM) *[Hamerman and Lanier, 2006, Siragam et al, 2006].* Therapeutic mAbs demand the

presence of functioning Fc region to suppress tumor invasion and to raise survival rates in mouse models. Thus, because glycosylation is an essential factor for the functions of human IgG , now, new strategy is adopting conjugation MAB with certain efficient glycoforms for more positive results *[Clynes et al, 1998].*

In my study, solamargine will be conjugated to the glycosylated Fc portion from its steroidal backbone, so that its functioning rhamnose terminal end is free and will be bound to gallium particles (discussed later in the methodology). By that way monoclonal antibody will act directly on malignant cells facilitating the Cytotoxic T cell function. Cancer has the ability to resist monoclonal antibodies by genetic polymorphism (discussed before) and suppression of host immunity (cytotoxic T cells). Cancer immunosuppression is triggered by tumor-derived soluble factors (TDSFs), like interleukin-10 (IL-10), transforming growth factor-b (TGF-b) and vascular endothelial growth factor (VEGF), and that spreads, starting from the primary tumour site reaching to secondary lymphoid organs and peripheral vascularity *[Zou, 2005, Yang, 2004].* Tumor derived VEGF is considered a powerful chemoattractant that initiates migration of immature myeloid cells (iMCs) from the bone marrow into peripheral vessels, where they are attracted to the primary tumor site by the action of chemokines and chemokine receptors *[Kusmartsev and Gabrilovich, 2002].* The iMCs, that entail immature dendritic cells (iDCs) and macrophages, have functional and biochemical remodelling within the tumor microenvironment into tumor-associated iDCs (TiDCs) and tumor- associated macrophages (TAMs) that are recruited to regional lymph nodes, spleen and peripheral circulation for immune evasion. The immunosuppressive iMCs and increased level of reactive oxygen species (ROS) suppress T-cell activation by specific tumor mechanism *[Kusmartsev et al, 2004].* Also the deficient clearance of apoptotic cells triggers formation of anti-DNA-antibodies creating pseudo- autoimmune response against host antigens. In a result pro-inflammatory response appears that increases tumor progression *[Kim et al, 2005].* High levels of auto-antibodies and iDCs stimulate production of CD4+ CD25+ regulatory T cells (Tregs) that suppress T-cell function. iMCs induce their immunosuppressive effect by stimulation of indoleamine 2,3-dioxygenase (IDO) (an enzyme responsible for tryptophan metabolism, tryptophan is needed for T-cell proliferation *[Munn et al 1999]* and ArgI (an enzyme responsible for L-arginine metabolism to ornithine and urea, and the polyamine oxidation from ornithine inhibits IL-2 production, that in a result suppresses T-cell proliferation *[Flescher et al, 1989]* by the help of IL-10 and TGF-b. The final result is production (ROS) that reduce the proliferation of T-cells *[Zea et al, 2005].*

c) *Role of gallium compound (within the solamargine–gallium compound)*

Gallium was chosen due to its role in tumor inhibition besides increased bioavailability and efficacy. Also prolonged presence of gallium intracellular raises its cytotoxicity level *[Rasey et al, 1982].* Selectivity for malignant cells is one of gallium advantages. Ga atoms have the ability to combine to DNA phosphate, constituting a stable complex. Ga compete with magnesium for DNA binding especially affinity of Ga for DNA is 100 times more than of magnesium *[Manfait and Collery 1984]* Ga forms transferrin- Ga complex after favorable binding with transferrin that results in DNA synthesis inhibition by its action on ribonucleotide reductase *[Chitambar et al, 1988].* Ga suppresses biosynthesis pathways within the cell and suppress protein synthesis *[Aoki et al, 1990].* The impact of Ga in affection of cell membrane permeability could be explained by changing the cell membrane potential, modulationof electric charges at the protein synthesis *[Collery et al, 1994].* Ga triggers efflux of calcium from mitochondria which is a necessary starting step for apoptosis *[Gogvadze et al, 1996].* Ga triggers the collagen and fibronectin synthesis *[Bockman et al, 1993]* which might illuminate the cause of the tumor fibrosis after long term administration *[Collery et al, 1986].* Ga is involved in intracellular oxidative stress, with a reduction in the ratio of cellular glutathione reduced form (GSH) on glutathione oxidized form (GSSG), an elevation in metallothionein (MT) and in hemeoxygenase-1 (HO-1) gene expression *[Yang and Chitambar, 2008].* Gallium salicylate *(fig.5)* (tetrakis(1-octanol) *tris*(5-aminosalicylate) gallium(III)) *[Ismail et al, 2006]* has anti-inflammatory, antitumor *[Perugini et al, 2000]* and antiangiogenic characteristics *[Borthwick et al, 2006]* besides the ability to suppress cancer cell progression *[Murono et al, 2000].* Salicylates can reduce platinum- based drugs toxicity *[Li et al, 2002]* and the irradiation toxicity *[Soderberg et al, 1988],* and it can increase chemosensitivity to anticancer drugs *[McCarty and Block, 2006].*

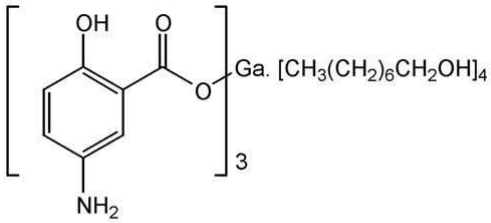



*Figure (5):* Structure of tetrakis(1-octanol) tris(5-aminosalicylate) gallium(III)

*d) Brief illustration of solamargine-gallium advantages when conjugated to monoclonal antibody*

1- Increased uptake by malignant cells due high the glycoside content (Warburg- effect base mechanism)
2- Solamargine anti-cancer properties: selectivity and cytotoxicity especially the MAB will bind to solamargine within its steroidal backbone allowing the rhamnose moiety facing cancer cells.
3- Monoclonal antibody glycosylation: solamargine attached to monoclonal antibody will direct it to malignant cell directly facilitating targeting cytotoxic T cells to cancer cells. Also solamargine addition site (Asn 297) (the same site of attachment monoclonal antibody with T cells) will not change the flexibility of Monoclonal antibody. In other words the hinge region of MAB can move with flexibility to attach Solamargine glycoside on tumor cells directly. Also receptor-mediated endocytosis of the modified MAB can occur by binding MAB Fc portion to FcγR expressed on malignant cells. It was observed by flow cytometry, polymerase chain reaction and sequence analysis that FcγR is expressed on malignant cells. Malignant cell FcγR can form complexes with tumor shed antigens and anti-shed tumor antibodies that augment cancer cell proliferation. So we can conclude that modified MAB has competitive antagonism role with tumor shed antigens and anti-shed tumor antibodies on binding to malignant cell FcγR *(Nelson et al, 2001).*
4- There will be three targeting effect or domains within the MAB –(solamargine-Ga) compound. The first is the Fab region of monoclonal antibody itself that antagonizes tumor signaling pathways, the second is solamargine and the third is gallium atoms (solamargine salicylate) that will be bound via its octanol domain to solamargine rhamnose moiety using almond β-glucosidase facing cancer cells.
5- Also the immunosuppressive mechanism of tumor can be compensated by the presence of both gallium and solamargine.
6- Anti-cancer properties of gallium atoms as discussed before.
7- Easy follow up for the tumor size: binding gallium atoms within the cancer will facilitate follow up imaging because gallium used in PTEN scan *[Mikuš et al, 2014].*

*e) Chemical and biochemical steps for formation monoclonal antibody conjugated with gallium-containing solamargine*

1. *Oxidation of methyl β D-glucoside* Methyl β-d-glucoside, one of the short-chain alkyl glucosides, has been used to synthesize long-chain alkyl glucosides by transacetalization *[Rather et al, 2012].* That reaction can be done by computer modeling with the GRI-Mech 1,2 reaction mechanism and theoretical calculation by the help of the RRKM master equation formalism *[C, -L Yu et al, 1995].* The yield of that reaction will be $CH_2O$ β D-glucoside. *(Reaction 1)*

*Reaction (1):* oxidation of methyl β D-glucoside

2. *Binding CH2O beta D-glucoside to steroid backbone of solamargine (ethanol) by dehydration reaction* (removal water molecule; OH from CH2O beta D- glucoside and (H) atom from the ethanol group of solamargine with the help of sulferic acid) to form D- glucose-CH-CH2-steroidal backbone of *solamargine*-Rhamnose moiety *(reaction2).* The aim of that reaction to yield solamargine with rhamnose free group, also bound to glucose on its steroidal backbone.

*Reaction (2):* reaction between solamargine and $CH_2O$ β D- glucoside by dehydratase enzyme to form D-glucose-CH-$CH_2$-steroidal backbone of solamargine-Rhamnose moiety

3. *Monoclonal antibodies production and glycosylation using CHO-S cells culture*

By the help of PiggyBac (PB) as transposon to carry out the integration of transgenes into the mammalian cells genome *[Wilson et al, 2007].* The PB transposon system can be of one or more transposon donor vectors, that express the transgene(s) and a helper vector encoding the PBase enzyme *[Balasubramanian et al, 2015].* The pB513B1 donor vector and pB200A helper vector will be brought. For construction a dual promoter vector, LC and BGH polyA sequences will be PCR amplified from the pUC-LC and pTracer-CMV2 vectors respectively. After cloning -into an intermediate vector, they will be cloned into pB513B1 by the help of EcoRI/ BamHI enzymes, and pBLP vector

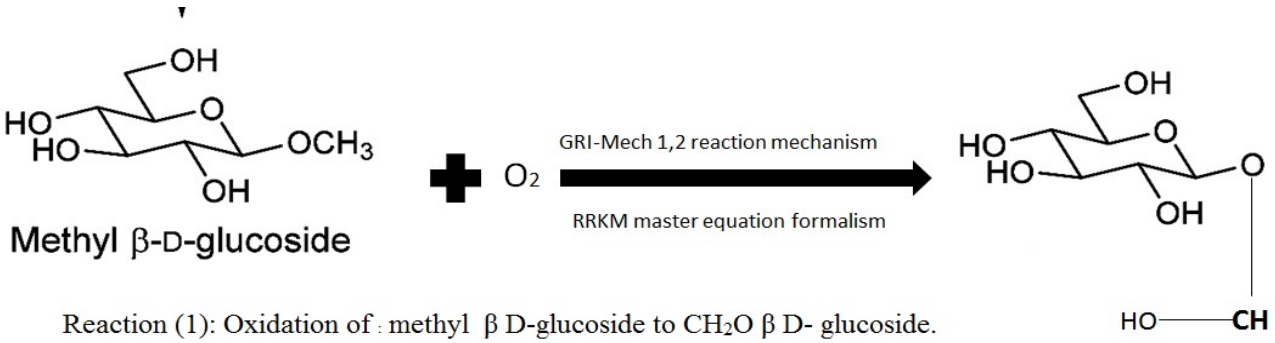
will be obtained. CMV-HC sequence will be sub-cloned from the pTracer-HC vector, into the pBPL vector by BglII/NotI to yield pBLPCH final construct. LC-IRES-HC and LC-F2A-HC (F2A; furin-containing 2A peptide sequence) fragments. LC-IRES-HC containing vector will be digested with NheI/NotI and the resulted fragment attached to the pB513B1 to produce pBLIH donor vector. LC-F2A-HC- cloned into pB513B1 by XbaI/NotI enzymes and pBL2AH will be resulted *[Ahmadi et al 2017].* Also another vector containing amplified sequence of glucosyltransferase enzyme will be prepared and injected into culture media cells. Then using suspension adopted CHO-S cells culture and (by the regular conditions and steps for purification) *[Ahmadi et al 2017]* and solamargine-glucose (formed in step 2), glycosylated monoclonal antibodies will be produced (rhamnose moiety is still not bound) *(reaction 3).*

*Reaction (3):* Purification of glycosylated monoclonal antibodies

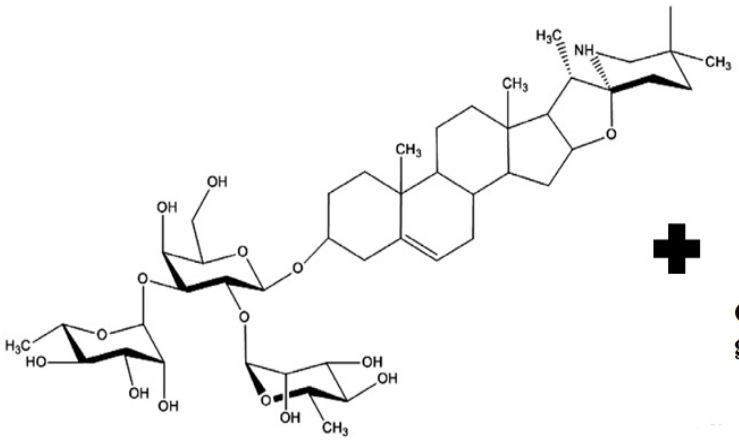
4. *Formation the final form (Monoclonal antibody-solamargine gallium salicylate)* tetrakis (1-octanol) tris (5-aminosalicylate) gallium(III) is the target gallium compound. Its octanol component will be reacted with D-glucopyranose of solamargine rhamnose moiety within the purified glycosylated monoclonal antibody by almond β-glucosidase enzyme (reaction 4). *(Mladenoska, 2016)* The substrates tetrakis (1-octanol) tris(5-aminosalicylate) gallium (III) and the purified glycosylated monoclonal antibody will be dissolved in octanol (dried with 3 Å molecule sieves) to a concentration of 10 mmol/L. The reactions will be done in closed glass vials, in an oven at 50 °C (IgG is denaturated irreversibly at temperature higher than 65° *[Akazawa-Ogawa et 2018]* and completely loses its antigen-binding activity after heat treatment for several minutes at 90 ° *[Akazawa-Ogawa et al, 2014]*, with vigorous shaking at 800 rpm. At different reaction times, samples will be withdrawn from the reaction mixture, put in the freezer for 30 min (monocloanal antibody can be stored in -20 ° without affecting its function *[Johnson, 2012],* diluted with mobile phase, and analyzed by HPLC and spectrophotometer.

*Reaction (4):* Synthesis of gallium octyl β-glucoside (glucoside: solamargine glucosyl monoclonal antibody).

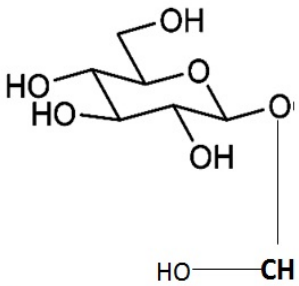
Then the efficacy of the modified monoclonal antibody can be compared with the original form of the same monoclonal antibody type, for example comparing cetuximab solamargine –gallium MAB with the efficacy of Cetuximab on epidermal growth factor expressing cancer cell lines such as colorectal cancer cell lines Cac0-2, DLD-1, HCT116 and HT-29.



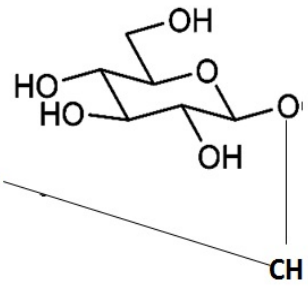

Reaction (1): Oxidation of : methyl β D-glucoside to $CH_2O$ β D- glucoside.



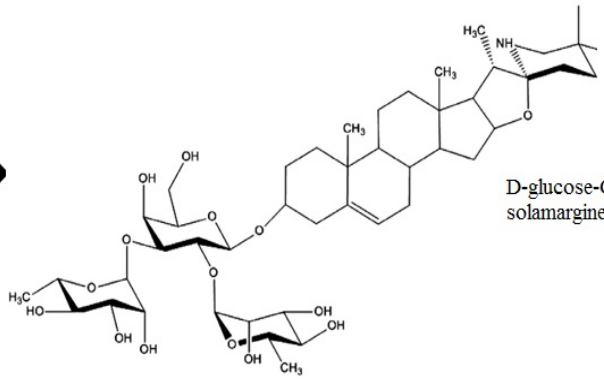
Solamargine



$CH_2O$ β D-glucoside



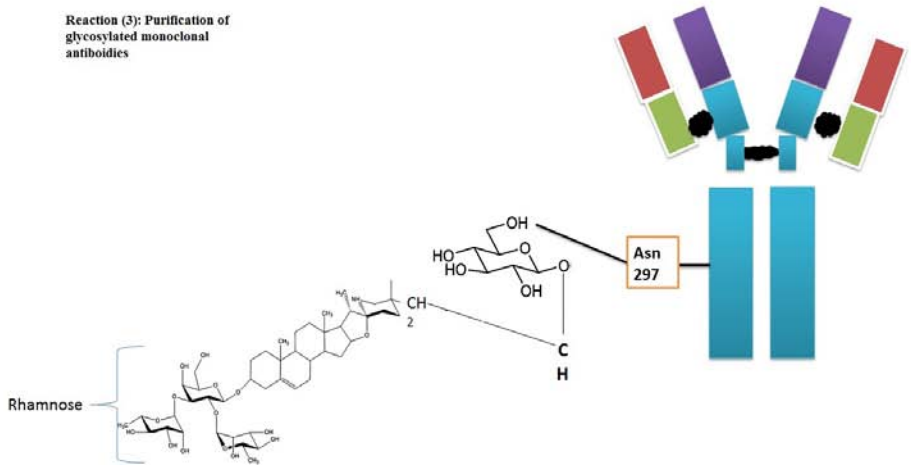
Dehydratase enzyme

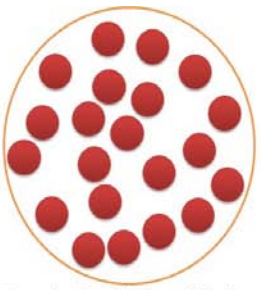


D-glucose-CH-$CH_2$-steroidal backbone of solamargine-Rhamnose

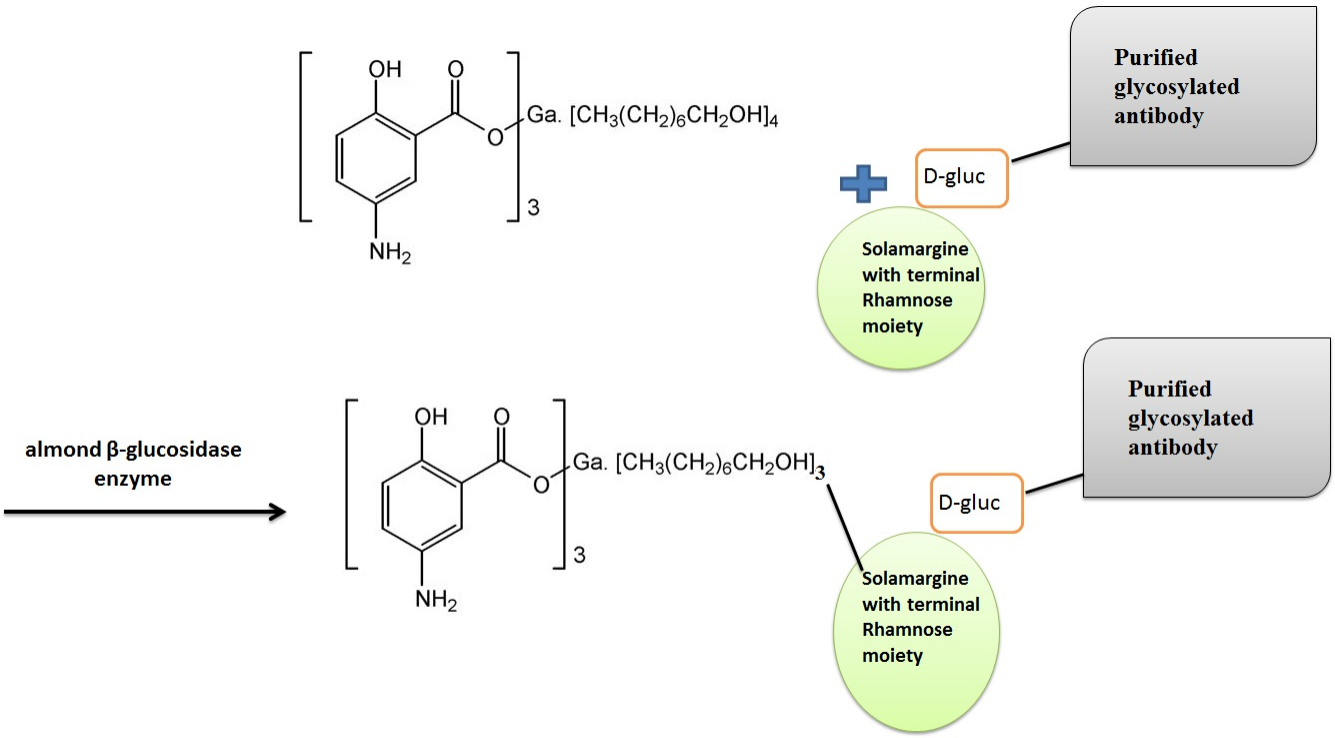
**Reaction (2)**

**Reaction (3): Purification of glycosylated monoclonal antiboidies**



Monoclonal antibody purification using CHO cell and glycosylation with glucose residue of D-glucose-CH-$CH_2$-steroidal backbone of solamargine-Rhamnose moiety by glucosyltransferase




## III. Conclusion

Modification of monoclonal antibody with gallium containing solamargine can be a general modification to different types of monoclonal antibodies because it is conjugated on Asn 297 which is a fixed structure to all monoclonal antibodies. That modified form can be easily targeted to cancer cells then endocytosis occurs after binding to malignant cell FcγR. Also inhibition the signaling pathway by the action of MAB Fab region will facilitate the suppressive effect of both gallium and solamargine. Besides that, Fab region of MAB can be a targeting structure to direct solamargine and gallium towards tumor cells. On the other side, cancer cells will be suppressed by the modified form of MAB by three components in the same time, MAB itself, gallium and solamargine. By that way, tumor resistance even by genetic polymorphism or immunosuppression of T cells will be markedly affected by the modified MAB if compared to the unmodified one.

*Conflict of Interest*

Author declares no conflict of interest about the article review.

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