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A REVIEW ON BIOCHEMICAL AND THERAPEUTIC ASPECTS OF ARGINASE

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ABSTRACT

One of the prime candidates in the treatment of debilitating human cancers includes group referred to as anticancer enzymes which acts by nutritional starvation therapy. The antitumor activity of these enzymes finds effective in countering wide range of cancers. Nutritional starvation of cancer cells have been tried by many researchers and have been found to be successful in a few cases. *Arginase* comes under this category. *Arginase* was proved to be effective in treatment of human hepatocellular carcinoma, melanoma and prostatic cancer. Apart from anticancer activity, arginase was found to be useful in the treatment of acute neurological disorder, allergic asthma and rheumatoid arthritis. The present review focuses on the isolation, production ,assay and media optimization of *arginase* enzyme and its therapeutic uses.

1. INTRODUCTION

Cancer is a disease characterized by abnormal proliferation of cells. It is one of the leading causes of death in the world, and it is of serious problem to people living in both the developing and developed nations [1,2,3]. Both benign and malignant tumors manifest as uncontrolled proliferation, but the latter are distinguished by their capacity to dedifferentiate, their invasiveness and their ability to metastasise. Cancer can be cured without much problem if it is diagnosed at the earlier stages. The treatments include chemotherapy, radiation and other forms of therapy. Every therapy has its own advantages and disadvantages. Nutritional starvation of cancer cells have been tried by many researchers and have been found to be successful in a few cases. Cancer cells have absolute requirements for nutrition, a few of its nutrients it can synthesize and for a few others it depends on the host cell. Amino acids like methionine and arginine come under these categories [4,5].If the cancer cells are deprived of these amino acids, they starve to death, since they can't synthesize these amino acids [6]. Nutritional starvation can be done by two ways, one by controlling the dietary intake of these amino acids and the other by decreasing the serum concentration of these amino acids. A number of enzymes are available which act by the same mechanisms to treat cancers. Examples : *asparaginases, glutaminases, arginases, arginine deaminase* etc. Microorganisms are considered as potential sources of these enzymes . Microbial anticancer enzymes are of considerable interest in view of their specific activity and stability at biological pH. The present review focusses on the isolation ,purification, assay and optimization of anticancer enzymes from microbial sources.

L-Arginase: Arginase (EC 3.5.3.1) is a 105 kD homotrimeric enzyme which requires an intact binuclear manganese cluster for its catalytic activity [7].*Arginase* is the terminal enzyme of the urea cycle among the six other enzymes. Interest in the *arginases* as possible regulatory enzymes is growing because of their potential for regulating the availability of arginine for the synthesis of NO, polyamines, agmatine, proline and glutamate [8].

Arginase was discovered in mammalian liver by Kossel & Dakin (1904) who showed that the products of its action are ornithine and urea. Since then, the enzyme has attracted a great deal of interest from many points of view. The enzyme has been found to exist in two forms that have evolved with differing tissue distributions, metabolic functions and subcellular locations in mammals [9]. The cytosolic form, *Arginase I* is found predominantly in the liver or hepatic cells and is important in ureogenesis. *Arginase II* is a mitochondrial enzyme that is extrahepatic and more widely distributed in numerous tissues, for example, kidney, and skeletal muscle [10]. It may be found at lower levels in macrophages, lactating mammary glands, and brain. Important roles of *Arginase II* have been reported to be the biosynthesis of polyamines, the amino acid ornithine, proline and glutamate and in the inflammatory process. Genetic "knockout" experiments suggest that *arginase II* functions in L- arginine homeostasis by regulating L-arginine concentrations for cellular biosynthetic reactions such as nitric oxide biosynthesis. The human type I and type II *arginases* are related by 58% sequence identity and are immunologically distinct.

Mechanism of Action: The *arginases* catalyse the divalent cation-dependent hydrolysis of L-arginine to form the non-protein amino acid L-ornithine and urea [11,12].

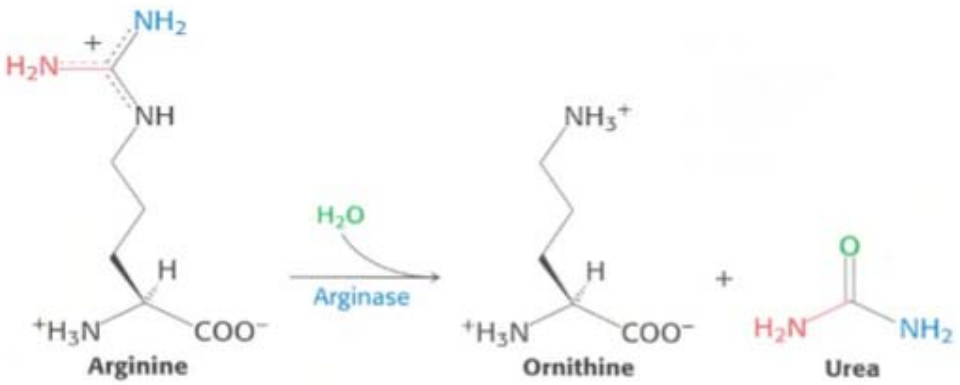

Figure 1 : The arginase reaction.

In the organisms containing *urease* ,urea is further converted to ammonia and carbondioxide.

Urea -------*urease*----------> NH_4^+ + CO_2

OCCURRENCE AND DISTRIBUTION:

Sources of *L-arginase :* *Arginase* apart from being ubiquitously present in mammalian tissues has also been characterized from various worms, molluscs, fishes, bacteria, fungi, yeast, actinomycetes, algae and plants.

Table no: 1

Bacteria	*Mycobacteria*	[13]
	Proteus spp	[14]
	T.aquaticus	[15]
	Agrobacterium-Rhizobium group	[16]
	Cyanobacterium Aphanocapsa	[17]
	Cyanobacteria,	[18]
	Bacillus licheniformis	[18]
	Bacillus subtilis 168	[19]
	Streptomyces spp.	[20]
	Streptomyces calvuligerus	[21]
	Streptomyces calvuligerus	[22]
	Cyanobacterium Anabaena cycadeae	[23]
	Thermophile Bacillus caldovelox	[24]
	Rhodobacter capsulatus E1F1	[25]
	Bacillus anthracis	[26]
	Chlamydia pneumoniae	[27]
Protozoa:	*Leishmania, Crithidia and Blastocridhidia*	[28]
	Entamoeba histolytica	[29]
	Plasmodium falciparum	[30]

Fungi	*Fungi: Neurospora crassa*	31
	Aspergillus nidulans	32
	Trichoderma sp.	33
	Agaricus bisporus	34
Lichens	*Evernia prunastri*	35
	Evernia prunastri and Xanthoria parietina thalli	36
	Peltigera canina	37
	Leptogium corniculatum	38
Yeast	*Saccharomyces cerevisiae*	39
	Schizosaccharomyces	40
Plant sources	*Lathyrus sativus*	41
	pumpkin seeds	42
	grapevine- Vitis vinifera	43
	Arachis hypogea	44
	Canavalia lineata	45
	Soybean	46
	Jack bean (Canavalia ensiformis)	47
	kiwifruit vines- Actinidia deliciosa	48
	, ginseng (Panax ginseng	49
	loblolly pine (Pinus taeda L.)	50
	Saccharum officinarum cv. Mayari	51
	tobacco	52
	Lycopersicon esculatum (tomato)	53
	Quercus ilex	54
	Vigna catjang	55
Insects	*Hyalomma dromedarii*	56
	Drosophila	57
	Bombyx mori- the silk worm	58
Sea organisms	*Larva of Phoronis pallida*	59
	African snail-Achatina fulica	60
	Protopterus aethiopicus and Protopterus annectens	61
	South American fish pacu (Piaractus mesopotamicus)	61
	South American fish pacu (Piaractus mesopotamicus)	62
	Sparus aurata	63
	Antarctic fish Notothenia rossii and Notothenia neglecta	64
	Pacific spiney dogfish shark Squalus acanthias	65
Mammals	*Salamandra salamandra*	66
	Genypterus maculatus	67
	Mus booduga	68
	Raja erinacea	69
	(Felis catus)	70
	Rana temporaria	71

Production, Characterisation & Purification of *arginase* from various sources: Numerous reports regarding the characterization of *arginase* & its purification ranging from microbial sources to vertebrate and invertebrate sources are accessible in scientific literature.

Production of *arginase* : Arginine has been reported to be utilized as a nitrogen source in *Neurospora crassa* [72]. Mora et al., grew *Neurospora crassa* in minimal medium supplemented with 1.5% sucrose and reported that arginine synthesised from exogenous citrulline was not effectively utilized as exogenous arginine due to feedback inhibition of *arginase* in vivo and in vitro by arginine pool formed from citrulline [73]. *Saccharomyces cerevisiae ATCC 9763* was cultured aerobically at 30°C in defined media containing arginine as a sole source of nitrogen. After 5 hrs of growth, *arginase* was extracted by sonication and partially purified by gel filtration [74]. Most of the arginine pool in *Neurospora* is available inside the vesicle and lesser arginine concentration is found to be present in cytosol in cells growing in minimal medium [75]. In arginine supplemented medium, the cytosolic pool dramatically increases resulting in induction of catabolic enzyme *arginase* as well as rapid catabolism of arginine. Vaca and Mora tested the effect of various nitrogen sources on arginase production in a *N. crassa* mutant ure-1 lacking urease activity and found that arginase was hyperinduced with arginine as the nitrogen source whereas in the wild-type strain the induction was completely repressed by glutamine[76]. In *Bacillus licheniformis* the *arginase* pathway enzymes are subject to strong catabolite repression during growth on glucose while glutamine antagonizes the induction of *arginase* pathway. This effect occurs only in a media containing a good carbon source. In this organism glutamine is a better nitrogen source than arginine, glutamate or ammonia. Broman et al., demonstrated that *Bacillus licheniformis* has two pathways of arginine catabolism and in well aerated cultures the *arginase* route is present, and levels of catabolic ornithine carbamoyl transferase were low [77]. They also demonstrated that an *arginase* pathway deficient mutant, BL196, failed to grow on arginine as a nitrogen source under these conditions.

The intensity of carbon source (glucose, citrate) catabolite repression of *arginase* seems to be much weaker in *B. subtilis*. In the *arginase* pathway in the *B. subtilis* strain used by Baumberg and Harwood repression of the pathway was by glutamine and, in addition, ammonia while ornithine and citrulline were useful ininduction of the enzyme [20]. In the genus *Agrobacterium*, arginine may be used as a nitrogen source by an inducible *arginase* and a constitutive urease. Many *Agrobacterium* strains are also able to use arginine and ornithine as carbon source. However, many *Agrobacterium* strains unable to grow on arginine or ornithine as a carbon source display this ability when they harbor a wild-type octopine or nopaline Ti plasmid [78].Synechcoystis sp. PCC 6803 were grown axenically in BG11 (nitrate containing) medium [79].Cultures were grown 30°C in light with shaking (80-90 rpm) for liquid cultures and supplemented with 1mM filter sterilized L-arginine. Drainas and Weiss (1980) studied the effect of various carbon sources on *arginase* activity in *Neurospora crassa* and found that basal and induced levels of *arginase* were reduced in the following order sucrose, acetate, glycerol and ethanol with regard to carbon sources used [80]. They also reported that arginine pools were similar regardless of carbon source in mycelia grown in arginine-supplemented medium and arginine degradation was proportional to level of *arginase* in both sucrose and glycerol grown mycelia suggesting a possible carbon metabolite effect on arginine metabolic enzyme genes in the fungus. Schreier et al., (1982) studied the regulation of inducible nitrogen catabolic enzyme *arginase* in *B. licheniformis* stating that although levels of enzyme were nitrogen source (Ammonia, Glutamine, Urea, Arginine, Ornithine) dependent its induction could still occur even in presence of preferred nitrogen sources [81]. *Neurospora crassa* strain was grown at 30°C in Vogel's minimal medium (VM) with 1.5% sucrose as carbon source. Initially it was supplied with 5mM arginine, 1mM histidine or NH_4NO_3,

later these were replaced with 5mM arginine which acted as nitrogen source. Liquid cultures were inoculated with 106 conidia per ml of media in baffled flasks and shaken at 250rpm [82]. Two forms of arginine with differing molecular weights i.e., 36 kDa and 41 kDa were produced in unsupplemented and arginine-supplemented media repectively.

Purification and kinetic characterization of arginase: Rabbit liver *arginase* was purified by Breitburd and Orth by chromatographic techniques of DEAE cellulose and Sephadex G-200 [83].It was strongly inhibited by EDTA and lost activity in absence of Mn2+ ions. SDS-PAGE showed that enzyme had a subunit molecularweight of 36,500, kDa increasing probability of its tetrameric nature. Molecular characteristics of chicken liver *arginase* were accounted by Grazi and Magri [84]. Purification and properties of rat kidney *arginase* were studied by Kaysen and Strecker showing the enzyme was highly stable at high temperatures such as 60^{o}C and had a Km value of 18 mM and was strongly inhibited by borate and L-ornithine [85].Tarrab et al., studied molecular isoforms of rabbit liver *arginase* by purifying them in a sequential manner by CM cellulose chromatography [86]. Purification studies of *arginases* from human-leukemic lymphocytes and granulocytes were done by Reyero and Dorner employing purification procedure of acetone extraction, ammonium sulphate precipitation, DEAE-cellulose, CM-Sephadex chromatography and gel filtration on Bio-Gel A [87].Both the *arginase* proteins were strongly basic with pI values between 9.25 and 9.35. Rat small intestinal *arginase* was purified by Sephadex -100 filtration and shown to have optimum pH of 10.0 and Km of 19 mM by Fujimoto et al [88]. The enzyme was almost completely inactivated by treatment with EDTA. The isoforms differed in their kinetic and pH behavior. *Arginase* from human liver and erythrocytes was isolated by Beruter et al., and was purified using DEAE-cellulose chromatography followed by gel filtration on Sephadex G-200 [89]. The human liver *arginase* had a molecular weight of was 107, 000 and its Km for arginine was 10.5 mM. The properties of human heart *arginase* studied by Baranczyk et al., revealed its Km to be 5 mM and molecular weight of about 30, 000 kDa with the enzyme having strong dependence on Mn2+ ions for its activity [90].

Gopalakrishna and Agarajan reported the purification of arginase from rat fibrosarcoma by heat treatment followed by DEAE-cellulose chromatography and gel filtration on Sephadex G-100 and accounted that the Km for arginine was 11 mM and had a pH optimum of 10 [91]. Purification and physical properties of *Arginase* from *Xenopus laevis* liver were studied by Peiser and Balinsky using procedures of heat treatment, acetone fractionation, and isoelectric focusing and its molecular subunit weight was determined by gel filtration on Sephadex G200 and found to be 76,000 daltons [92]. *Arginase* was obtained from Iris hollandica bulbs and purified approximately using DEAE-Sephacel chromatography, aminohexyl-Sepharose 4B chromatography and gel filtration on Ultrogel AcA 34 by Boutin (1982) [93]. Constitutive *arginase* from *Evernia prunastri thallus* was purified 920-fold and is activated by endogenous L-arginine [94]. Borkovich and Weiss in 1987 reported that *Neurospora crassa arginase* has a subunit weight of 38,300 determined by SDS-PAGE [31]. The enzyme exhibited hyperbolic kinetics at pH 9.5 with an apparent Km for arginine of 131mM. Antiserum was prepared against the purified enzyme and two proteins in the extracts of *S. cerevisiae* were detected that were weakly cross- reactive with the antiserum. Two forms of *arginase* were isolated from human erythrocytes and their immunological properties were studied by Kedra et al [95]. Later, Ikemoto et al., (1989) developed an efficient method involving hydrophobic chromatography and immunoaffinity chromatography for purification of human erythrocyte *arginase* [96]. Patil et al., (1990) gave a high recovery protocol for purification of ox erythrocyte *arginase* involving heat treatment, CM and DEAE-Sepharose chromatography, arginine AH-Sepharose chromtagraphy and molecular sieving through Biogel P-150 [97]. The optimum pH was 11.5 and temperature was 55^{o}C for purified enzyme. Soyabean Glycine max

axes *arginase* was purified by Kang and Cho and its properties were studied [46]. The enzyme purification procedure followed was ammonium sulphate precipitation followed by chromatography on Sephadex G- 200, DEAE Sephacel, Hydroxypatite and Arginine-Sepharose 4B affinity column. The enzyme had a Km of 83 mM and pH optimum of 9.5 with a molecular weight of 240, 000 obtained by pore-gradient elctrophoresis. Purification of recombinant human liver *arginase* expressed in *E. coli* was described by Ikemoto et al., by chromatographies on CM-Sephadex G-150, DEAE-cellulose and Sephadex G-150 followed by preparative gel electrophoresis [98]. The purified enzyme was a monomer of molecular weight 35,000 kDa. Singh and Singh, purified liver *arginase* from teleostean fish-Clarias batrachus having Km of 15.38 mM for arginine and an optimum pH of 9.5 [99]. Ornithine and leucine act as competitive inhibitors whereas valine and isoleucine act as non-competitive inhibitors with respect to L-arginine as substrate. Purification of *arginase* from *S. cerevisiae* was done by Sepharose chromatography and TSK-G3000 gel filtration followed by its characterization (Green et al., 1989) which revealed a Km of 15.7 mM for arginine and requirement of Mn2+ ions for activity. CD spectra analysis showed significant spectral changes from removal of bound metal and dialysis against EDTA [100].

A thermostable *arginase* from the extreme thermophile *Bacillus caldovelox* was purified by chromatographic techniques and its kinetic properties were studied in 1991 by Patchett et al [24]. Its activity is optimal at pH 9 and temperature of 60°C. The Km for arginine is 3.4 mM. *Arginase* obtained from the phototrophic bacterium- *Rhodobacter capsulatus* E1F1 was purified and studied for its properties. The molecular parameters and kinetic constants of *Rhodobacter arginase* resembled the *Saccharomyces cerevisiae* enzyme rather than those of bacterial *arginases* [25]. Extrahepatic arginase (ArgII) was isolated from rat mammary gland and its properties were investigated by Jenkinson and Grigor (1994) [101]. This enzyme had a pH optima of 10 and Km of 12-14mM. Purification of human hepatic *arginase* and its manganese (II) - dependent and pH-dependent interconversion between active and inactive forms was described by Kuhn et al., (1995) who showed that both the maximal velocity of catalysis and the Km toward arginine were markedly pH dependent in the physiological range [102]. In situ characterization of *Helicobacter pylori arginase* employing NMR spectroscopy, spectrophotometry, radiotracer analysis and protein purification techniques was done by Mendz et al [103]. A Km of 22+/- 33mM was determined for the enzyme activity and differences of Vmax were observed between strains. Divalent cations stimulated *arginase* activity & most potent activators were Co+2 > Ni+2 > Mn+2.

Recombinant human arginase II was expressed in *E. coli* and purified to homogeneity by Colleluori et al., (2001) [104]. The enzyme reportedly had a Km of 4.8 mM at physiological pH and exists primarily as a trimer. Borate was a non-competitor inhibitor of the enzyme and ornithine which is an inhibitor of *Arginase-I* is not an inhibitor of the type-II enzyme showing that isozyme selectivity occurs between both forms with regard to substrate and product as well as inhibitor binding. *Pinus taeda arginase* was purified by chromatographic separation on DE-52 cellulose, Matrex Green and arginine-linked Sepharose 4B and had a molecular mass of 140 kDa deduced by FPLC while the subunit size was shown to be 37 kDa observed by SDS-PAGE analysis [50]. *D-arginase* obtained from *Arthrobacter sp KUJ 8602* was purified by anion exchange chromatography using DEAE-Toyopearl 650M followed by hydroxyapatite chromatography and gel filtration chromatography with Superdex 200 [105]. The purified enzyme was proposed to be a homohexamer with each subunit of approximately 40, 000 determined by SDS-PAGE. It had an optimum pH of 9.5 and needed Zn2+ for activation instead of Mn2+. Bovine liver *arginase* as reported by Wheatley et al., (2003) is a remarkably heat resistant enzyme with a very long life on storage at 4°C in lyophilised form and is occasionally marginally more active at pH 7.2 than at pH

9.9 [137]. Helicobacter pylori *arginase* expressed in *E. coli* was loaded onto polyproplylene columns containing nickel- nitrilotriacetic acid agarose resin for purification (McGee et al., 2004) [106]. The purified enzyme had significant activity with cobalt as cofactor and had acidic pH optima of 6.1. It was inhibited by low concentrations of reducing agents. Dabir et al., (2005) described the purification, properties and alternate substrate specificities of the enzyme from two different sources: Vigna catjang cotyledon and buffalo liver [55]. The Michealis-Menten constant for cotyledon arginase and hepatic arginase were found to be 42 mM & 2 mM respectively and their pH-optima were 10 & 9.2 respectively. Munder et al., (2005) have reported that arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity [107]. Two *arginases* Arg I and Arg II were separated from *Fasciola gigantica* by purification on a DEAE-Sepharose column as reported by Mohamed et al., (2005)[108]. Further purification was carried out for Arg II and it was shown that the enzyme was activated by Mn2+ and inhibited by Fe2+, Ca2+, Hg2+, Ni2+, Co2+ and Mg2+ ions. Purification of recombinant Plasmodium *falciparum arginase* was done on Superdex S-200 column and its activity was found to be dependent on Mn2+. Ni2+ when replaced with Mn2+ resulted in four-fold loss of activity [30]. Viator et al., (2008) reported the purification of recombinant *Bacillus anthracis arginase* expressed in E. coli XL1-Blue MRF' on *arginase* affinity columns [109].

Structure related studies

Most of the studies related with the structure of *arginase* are by means of studying the interaction of various inhibitor complexes with the substrate specific sites of the enzyme. Electron paramagnetic resonance (EPR) studies on rat liver arginase by Reczkowski and Ash revealed that fully Mn-activated *arginase* contains two Mn2+ ions per subunit and these form EPR spin-coupled binuclear centers [110.] The high resolution X-ray crystal structure of rat *arginase I* was provided by Kanyo et al., which illustrated that the enzyme is a 105 kDa homotrimer with each subunit of 35 kDa containing a spin-coupled binuclear manganese cluster critical for its activity located at the bottom of a ~15 Ao -deep active site cleft [111]. Bewley et al., studied the crystal structure of *arginase* structure of inactivated *Bacillus caldovelox arginase*-L-arginine complex which revealed that the structural basis for substrate and inhibitor specificity are an array of direct and water mediated hydrogen bonds saturating all four acceptor positions on α-carboxylase group and all three donor positions on α-amino group [112]. Cox et al., noted the binding of Nω-hydroxy-L- arginine (NOHA)-an intermediate of NO biosynthesis and modest inhibitor of the enzyme with rat *arginase I* which revealed that the Nω-hydroxyl group displaces the metal-bridging hydroxide ion and bridges the binuclear manganese cluster and that in the binding of rat *arginase I* and nor-NOHA the Nω-hydroxyl group of the inhibitor displaces the metal bridging hydroxide ion [113]. The structure for human kidney II type *arginase* is nearly identical to that of rat *arginase I* due to conservation of all metal ligands between the two sequences and its polypeptide fold is topologically identical to α/β fold of rat *arginase I* and hexameric *arginase* from *Bacillus caldovelox* [114]. Crystal structure of human arginase I at 1.29- angstrom resolution has been studied by Di-Costanzo et al., [115]. Inhibitor complexes of *arginase I* with inhibitors ABH and BEC were studied to know the inhibition of the enzyme in human and urine myeloid cells to study its effect on immune response. The role of the hyper- reactive histidine residue (H141) in rat liver *arginase* was studied by studying the structure of the enzyme by chemical modulation, mutagenesis and X-ray diffraction [116]. By the study, the H141 has been shown to possess conformational mobility supported by its proton shuttling role and proposed to be an acid/base catalyst by deprotonating the metal-bridging water molecule to generate the metal-bridging hydroxide nucleophile, and by protonating the amino group of the product to facilitate its departure. Di Costanzo et al., determined the X-ray crystal structure of a perdeuterated human *arginase I* complexed with 2(S)-

amino-6- boronohexanoic acid (ABH) at 1.90 A^{o} resolution and foud that the the perdeuteration did not cause any structural and functional changes when compared with the unlabeled enzyme thus rendering the perdeuterated crystals suitable for neutron crystallographic study [117]. Dowling et al., (2010) have reported the 2.15 Å resolution crystal structure of arginase from the cerebral malarial parasite *Plasmodium falciparum* in complex with the boronic acid inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) - the first report of crystal structure of a parasitic *arginase* [118]. Inhibition studies with the enzyme structure lend important insights into the antimalarial therapy against liver-stage infection, and ABH may serve as a lead for the development of inhibitors.

Assay procedures for Arginase :

Numerous methods based on different principles for assaying *arginase* are available. Some of them are listed below:

Colorimetric Method: These methods are based on the colorimetric determination of urea or ornithine- the products of arginase reaction which can be assayed by the development of a colorimetric procedure. In the procedure of urea determination (Foster et al., 1971) - urea is determined using 2,3 – butanedione reagent (BUN reagent) [119]. This method has been reported for arginase assay (Basch, 1997) [120]. The colour is measured at 460 nm. In the method of Coulombe and Favreau, (1963) urea formed gives red colour on reaction with diacetyl monoxime and thiosemicarbazide in acid medium whose absorbance is measured at 535 nm [121]. In the method of Davis and Mora, (1968), the urea formed is assayed by reaction with α- isonitrosopropiphenone [122]. This procedure was originally devised for high accuracy urea determination in blood filtrates and urine by Archibald (1945) [123]. The absorbance change is read at 540 nm. A novel colorimetric assay was devised by Aminlari (1992) where determination of residual arginine, after its conversion with p-nitrophenyl glyoxal (PNPG) at pH 9.0 in the presence of sodium ascorbate was monitored [124]. The decrease in absorbance in the presence of arginase correlates with the enzyme activity. A colorimetric microplate assay for *arginase* was recently developed by Iyamu et al., (2008) for high-throughput analysis of *arginase* activity in vitro [125]. The method was a modified form of the Chinard reaction of ornithine-ninhydrin reaction.

Electrochemical Method:

Booker and Haslam (1974) used the following reaction sequence for assay of *arginase* by means of a cation NH_4^+ electrode that was used to sense the NH_3 liberated and immobilized urease was used [126.] From 1.6 to 16 U of *arginase* could be assayed in 10 min with a CV of 3%.

L-Arginine -----*L-Arginase*----> Urea + L-Ornithine

Urea --*urease*--------> NH_4^+ + CO_2.

Larsen et al. (1975) have described a kinetic method for *Arginase* measuring the NH_3 liberated with an air gap NH_3 electrode [127]. The above sequential reactions liberate NH_3 whose determination as NH_4^+ is done by the electrode. The determinations were based on monitoring the initial reaction rates of the selective release of ammoniacal nitrogen.

A tenfold excess of *urease* ensured direct proportionality between the rate of NH_3 production and the *arginase* activity. The coefficient of variation (CV) was 2.8%.

Spectrophotometric Method: Ward and Srere reported a novel *arginase* spectrophotometric assay by based on the principle that the absorbancy of *arginase* below 2100 Å is larger than the combined absorbancies of ornithine and urea [128]. A cleavage of arginine catalyzed by the enzyme thus results in a net decrease in absorbancy at these wavelengths, allowing a rapid and accurate assay for *arginase* activity. Ozer developed a new spectrophotometric assay for *arginase* in which the enzyme is coupled to urease and glutamate dehydrogenase and the decrease in absorbance at 340 nm due to the oxidation of NADPH is followed [129]. Han and Viola (2001) developed an alternative assay for *arginase* by synthesising an alternative substrate of *arginase* has been synthesized in which the bridging guanidium nitrogen has been replaced with a sulphur [130]. This thiol compound is a good substrate for *arginase*, leading to urea and 2-amino-5-mercaptovaleric acid which can be monitored with DTNB to produce a disulfide adduct and release 2-nitro-5-thiobenzoate as a chromophoric product.

Flourimetric Method: Orfanos et al., described a microfluorometric method for assay of *arginase* activity in dried bloodspots on filter paper [131]. The urea formed from the *arginase* reaction is determined flourometrically by oxidation of NADH to NAD^+ in a coupled kinetic reaction.

Conductimetric method: Macholan et al., have constructed a bioelectrode consisting of two parallel noble metal nets and a thin layer of gel- entrapped urease (for urea determination) and microbial cells or tissue slice (for *arginase* determination) for conductimetric measurements in tissue extracts [132].

Radioisotopic Method: This method was reported by Carulli et al., with an underlying principle of hydrolysis of labeled substrate (argininеguanido-14C) [133]. The labeled urea produced is hydrolyzed by urease with the measurement of released $14CO_2$ in a liquid scintillation counter as a measure of *arginase* activity. Advantages of the method reported were sensitivity and accuracy derived from the absence of interference by nonradioactive urea in the incubation mixture.

Volke et al., reported a simple and fast HPLC method with radiochemical detection for measurement of arginase activity by use of radiolabeled arginine [134]. de-Bono et al., (2007) performed radiochemical HPLC detection of arginine metabolism resulting in measurement of nitric oxide synthesis and *arginase* activity in vascular tissue [135].

Other methods: Bastone et al., carried out measurement of *arginase* along with argininosuccinate lyase both in liver and erythrocytes, by using a commercial amino acid analyzer [136]. The two different substrates used by both the enzymes give the similar product ornithine whose measurement is done by this method. Assay kits such as QuantiChromTM *Arginase* assay kit for determining *arginase* activity are commercially available in market.

ARGIINASE AND ITS DEVELOPMENT AS A CHEMOTHERAPEUTIC AGENT:

The use of *Arginase* as a potential chemotherapeutic agent has shown a lot of potential and promise. *Arginase* treatment of cultured HeLa, human diploid fibroblasts and L1210 cells proved to be as efficient as the use of AFM (Arginine Free Medium) by reducing arginine in the medium to micromolar levels within 5-30 minutes resulting in cell death in both the cultures [137]. Remission of hepatocellular carcinoma was achieved by arginine depletion through endogenous human hepatic *arginase* released from transhepatic arterial embolization [138].Using

state-of-the-art DNA technology, researchers of the Hong Kong Polytechnic University (PolyU) produced a human recombinant *arginase* as a novel method in the treatment of liver cancer by arginine depletion [139]. The combination of the recombinant *arginase* with an anti-neoplastic agent 5 flourouracil (5FU) for treatment of human malignancies was tested in nude mice bearing an ADI-resistant HCC xenograft and this treatment methodology was shown to be effective for arginine depletion [140]. BCT-100 pegylated recombinant human *arginase* manufactured by large scale fermentation of a recombinant *B. subtilis* strain LLC101 has been reported as a novel anti-melanoma agent for treatment of human melanoma cells as reported by Hsueh et al.[141]. Pegylation of recombinant human *arginase* (rhArg-peg 5000mw) produced in *B. subtilis* expression system was done and the pegylated enzyme has been shown to have in vitro and in vivo anti-proliferative potential and apoptotic activities in human hepatocellular carcinoma (HCC) by Cheng et al. [142]. While studies for conducting clinical trials of PEG BCT-100 are reported to be in process to assess its safety and efficacy in humans (http://clinicaltrialsfeeds.org/ clinical-trials/show/NCT00988195), recently further studies on pegylation of recombinant *arginase* conducted by the Cheng group by conjugation of rhARG with methoxypolyethylene glycol-succinimidyl propionate (mPEG-SPA 5,000) has been reported as having comparable anti-tumor efficacy to native rhArg1[143]. That the inhibition of human hepatocellular carcinoma by recombinant *arginase* (rhARG1) is by the inducing cell cycle arrest at the G2/M or S phase, possibly mediated by transcriptional modulation of cyclins and/or cyclin dependent kinases (CDKs) has been observed by Lam et al. [144]. This group has further proposed the use of rhArg1 alone or in combination with chemotherapeutic drugs for treatment of liver cancer.

In vitro cytotoxicity of human *arginase I* by replacing the two Mn2+ ions normally present in the enzyme with Co2+ significantly lowered the Km value of the enzyme, increased its serum stability and showed incredible ability to eliminate human hepatocellular carcinoma and melanoma cell lines proving it to be a capable new contender for treatment of L-Arg auxotrophic tumors [145]. A study conducted by Hernandez et al., published just a few months back in the prestigious journal 'Blood' reports of the potential therapeutic role of pegylated *Arginase I* in the treatment of adult patients with acute lymphoblastic T cell leukemia (T-ALL) through arginine depletion [146].

Recombinant Arginase

Development of recombinant *arginase* has been an intriguing subject of research worldwide. Molecular cloning and nucleotide sequence of cDNA for human liver *arginase* facilitated the investigation of the enzyme and gene structures and helped in elucidating the nature of mutation in argininemia. *Arginase* activity was detected in *Escherichia coli* cells transformed with the plasmid carrying lambda hARG6 cDNA insert by Haraguchi et al [147]. cDNA phARG6 for human liver *arginase* was used for expression of human liver *arginase* in *E. coli* strain KY1436 by Ikemoto et al [98]. This *E. coli* expressed human liver *arginase* had chemical, immunological and most catalytic properties indistinct from purified human erythrocyte *arginase*. The cloning, expression and crystallization of a thermostable *arginase* from the thermophilic bacterium '*Bacillus caldovelox*' has been discussed by Maria et al [148]. In this

study, the expression of recombinant *arginase* at high levels was achieved in E. coli using an inducible T7 RNA polymerase based system. Molecular cloning and nucleotide sequence of the *arginase* gene of *Bacillus brevis* .

TT02-08 and its expression in *Escherichia coli* has been described by Shimotohno et al [149].The *B.subtilis arginase* encoding rocF gene was cloned and expressed in *E.coli* K-12 for enhanced production of urea by *arginase* pathway by Tuchman et al [50]. Modulation of mRNA levels of liver *arginase* by insulin and vanadate in experimental diabetes has been studied by Salimuddin et al., (1999) where it was shown that an increase in *arginase* activity and mRNA levels in diabetes and decrease in treated animals may be due to the transcriptional regulation of *arginase* gene [151]. Kimura et al., transformed *E. coli* strain KY1436 by pTAA12 expression plasmid vector containing human liver-type *arginase* cDNA and developed an ELISA system by using this recombinant enzyme as an antigen for the detection of anti- *arginase* antibodies in patients with Autoimmune Hepatitis (AIH) [152]. The expression, purification and characterization of human type II *arginase* involving genetic engineering experiments to develop a recombinant human type II *arginase* expressed in *Escherichia coli* was done by Colleluori et al. [104].The recombinant enzyme was studied for its kinetic properties and inhibitor effects on it. The findings indicated that isozyme selectivity exists between type I and type II *arginases* for binding of substrate and products, as well as inhibitors. The rocF gene encoding *arginase* in *Helicobacter pylori* was purified and expressed in *E. coli* by McGee [106] and was found to confer *arginase* activity to *E. coli*. The transformed cells expressing *arginase* showed enhanced enzyme activity than the native *Helicobacter* enzyme. *Recombinant arginase* was developed by cloning DNA of *Plasmodium falciparum* (*Pfarginase*) into *E. coli BL21*. The recombinant enzyme had a Km of 13±2 mM, required Mn2+ for activity and had high thermal stability [30]. The RocF gene of *Bacillus anthracis* was cloned in *E. coli* for development of a recombinant enzyme. The metal preference of the enzyme was shifted at pH 6 from Ni>Co>Mn to Ni>Mn>Co at pH 9. A novel recombinant *B. subtilis* prophage strain LLC101 was constructed for recombinant enzyme production by Cheng et al [138]. The recombinant enzyme produced was used for treatment of human malignancies by subsequent pegylation [138,142,143]. A highly active recombinant *arginase* was obtained by expressing *arginase* gene from *Leishmania* (*Leishmania) amazonensis* in *E. coli BL21* (DE3) cells [153]. Antibody against the recombinant protein confirmed a glycosomal cellular localization of the enzyme in promastigotes. RhArg causing significant cytotoxicity in LNCaP, DU-145 and PC-3 prostate cancer cells was shown by Eddy C Hsueh etal .

Modification of Arginase: Immobilization & Chemical Modification

Attempts to increase the activity and stability of the enzyme by various techniques of immobilization and chemical modification have been made. Vanillin polymethacrylates (vanacryls) were used for immobilization of *arginase* by Brown and Joyeau . Carvajal et al., reported the immobilization of human liver *arginase* by attachment to nylon with glytarahldehyde as a cross-linking agent [154]. The immobilized enzyme tetrameric enzyme dissociated into monomers by treatment with EDTA. Influence of immobilization on solid matrix on rat liver *arginase* revealed that the immobilized enzyme was more resistant to effect

of inhibitors, denaturing agents such as SDS and chelating agents such as EDTA than free enzyme [155]. Bovine liver *arginase* was covalently immobilized by glutaraldehyde method to inner surface of Cuprophan hollow fibers of a conventional hemodialyzer by Rossi et al.[156]. The immobilization method did not harmfully affect the physical and mechanical properties of neither hollow fibers nor their hemocompatibility. Aguirre and Kasche, by covalently coupling the enzyme to Sepharose beads and dissociating the resulting matrix-bound tetramer by acid or EDTA treatment, obtained catalytically active monomer forms of immobilized rat liver *arginase* [157]. Savoca et al., studied the therapeutic effectiveness of *arginase* in cancer therapy on a Taper liver tumor and the L5178Y murine leukemia by modifying it by covalent attachment of polyethylene glycol. The pegylation of the enzyme increased its stability [158]. Beef liver *arginase* was modified by the covalent linking of monomethoxypolyethyleneglycol molecules by Visco et al [159]. The derivative enzyme had more convenient properties for a therapeutic use, such as increased structural stability, decreased digestion by proteolytoic enzymes and an expanded clearance time in rats. Veronese et al., discussed the immobilization of *arginase* by radiation induced polymerization of induced monomers [160]. Properties of *arginase* immobilized in a fibrin clot were studied by Diez et al. [37]. That chemical modification of rat liver *arginase* by N-bromosuccinamide leads to its inactivation was reported by Daghigh et al [161]. *Arginase* isolated from beef liver was covalently attached to a polyacrylamide bead support bearing carboxylic groups activated by a water-soluble carbodiimide [162].The pH optimum for the catalytic activity was pH 9.5 and apparent temperature maximum was 60° C. Immobilization markedly improved the conformational stability of the enzyme. An enzyme reactor for determination of L-arginine was described by Alonso et al., by immobilization on an epoxy resin matrix [163]. Pegylation has been reported to greatly improve the stability of the enzyme [137]. Pegylated recombinant human *arginase* has in vitro and in vivo anti-proliferative potential and apoptotic activities in human hepatocellular carcinoma by Cheng et al [138,142]. For studying thermodynamics of nor NOHA/*arginase* binding and the role of the reactive histidine residue *arginase* was immobilized on a chromatographic support by Bagnost et al.[164]. Pegylation of rhARG with methoxypolyethylene glycol-succinimidyl propionate (mPEG-SPA 5,000) proved to be the best for activity retention of the immobilized enzyme for use in arginine depletion as an anti-cancer therapy protocol[143]. *Leishmania arginase* was immobilized on a Ni2+ resin and its activation kinetics was studied by varying Mn2+ concentrations at a temperature of 23^{o}C. Conformational changes were observed when enzyme interacted with Ni2+ present in the column [165]. Konst et al., have reported the stabilization and immobilization of *B. subtilis arginase* on commercially available epoxy-activated supports out of which immobilization on Sepabeads EC-EP was most promising [166]. The immobilized enzyme was employed in the biobased production of nitrogen-containing chemicals as an alternative to the petrochemical production.

Arginase: The multilateral medical therapeutic

The enzyme has been found to possess profound therapeutic benefits in treatment of various physiological disorders in the body. Measurement of circulating *Arginase I* i.e., serum *arginase* levels have been used experimentally as rapid marker for liver injury [167]. *Arginase* has been found to be essential for the treatment of acute neurological disorders[168]. Ornithine, produced

by *arginase* is necessary for the production of collagen, is helpful in therapy of rheumatoid arthritis [169]. *Arginase* upregulates the synthesis of polyamines and proline via arginine hydrolysis thus, being necessary to provide compounds for cell proliferation and growth [170]. *Arginase* competes with NOS for endogenous arginine pools, this way its levels acts as an indirect regulator of penile and vaginal flow thus playing an important role in male and female sexual arousal [171].Upregulation of endogenous *arginase I* causes the activation of neural regeneration pathways, the reaction being mediated by polyamines and leading to novel roles of *arginase* in cell survival, regeneration and translation in the central nervous system [172]. Munder et al., reported that *arginase-I* is constitutively expressed in human granulocytes and participates in fungicidal activity by a novel antimicrobial effector pathway likely through arginine deprication in phagolysosome [173]. A deficiency of the liver enzymeresults in hyperargininemia- inherited in an autosomal recessive manner [174]. Raised arginase activity in serum of 85% patients suffering from colorectal cancer liver metastases (CRCLM) led to the conclusion that *Arginase* can be a useful marker for diagnosis of CRCLM [175]. Human granulocyte *arginase* has been reported to be a promising pharmacologic treatment to reverse unwanted immunosuppression by Munder et al [176]. Increase in psychological stress results in raising NO level which results in poor sperm quality. Thus, *arginase* levels are important to keep NO levels (via arginine degradation) in control for maintainence of semen quality[177]. *Arginase* has been shown to be effective for treatment of Hepatitis- B [139]. A finding that Hepatitis C virus targets overexpression of *arginase I* in hepatocarcinogenesis suggests that insights into this disease and its control may be through altering arginine metabolism by means of *arginase* activity alterations [178].

The *arginase* levels in NOS related processes prove to be important for pathogenesis of a host of diseases relevant to arginine-NO balance in the body. Due to *arginase* and nitric-oxid synthase (NOS) utilizing the same substrate arginine, a reciprocal regulation and metabolic balance exists between both of them for numerous processes that are dependent on these enzymes, occur inside the body. In rheumatoid arthritis patients a significant correlation between serum concentration of *arginase* protein and rheumatoid factor has been found suggesting that increased *arginase* production may play an important role in pathogenesis of this disease [49]. *Arginase I & II* levels rise remarkably in asthma and lung infection thus, showing the involvement of *arginase* in the pathology of the disease [179]. Measurements of plasma *arginase* activity provide a useful marker for underlying metabolic disorder and efficacy of treatment for asthma [180]. Increase in levels of arginase during Sickle cell disease makes it a candidate for pathogenesis of this disease [181]. The pathogenesis of cystic fibrosis has been reported to be *arginase* dependent since *arginase* levels are enhanced leading to depletion of arginine for NOS action to occur contributing to nitric oxide deficiency [182]. Because of its role in epidermal hyperproliferation by upregulating CAT-1 expression in psoriatic skin, *arginase* is important in pathophysiology of psoriasi [183]. Similarly, since nitric oxide is a chief vasodilator, high arginase activities have often been associated with heart ailments such as atherosclerosis, mycordial infarction and ischemia. *Arginase* has been shown to modulate myocardial contractility by nitric oxide synthase 1- dependent mechanism [184]. Higher *arginase* activity in lower Mg2+ ions have been observed in diabetic children by Bjelakovic et al.,

suggesting the pathophysiology of this disease could be understood to be related with increased arginine catabolism due to *arginase* action [185]. The *arginase*-NO synthase competition for arginine has led to various effects on airway hyperresponsiveness, bronchial obstruction and lung inflammation in asthma due to increase in *arginase* activity. Munder have reported *arginase* modulation to have a noteworthy role in the mammalian immune system due to L-arginine metabolism being regulated by it leading to immunosuppression by suppression of T-cell immune responses [186]. Maarsingh et al., and Munder have reported *arginase* to be a key enzyme in pathophysiology of allergic asthma opening novel therapeutic roles for asthma control through action of *arginase* inhibitors [187,188].

Table No: 2. Commercial available microbial arginase:

Name	Category	Company name	Application
Arginase	Arg1	U.S.Life Sciences	Suitable for use in ELISA, Western Blot, Immunoprecipitation and Conjugation. Other applications not tested

CONCLUSION

Microorganisms were considered to be potential sources of number of therapeutic enzymes like anticancer agents. Arginase can be produced commercially using these microorganisms in very less cost . Number of studies should be carried out and further steps has to be taken to promote therapeutic arginase in the market .

REFERENCES

1. Hail Jr N. Mitochondria: a novel target for the chemoprevention of cancer. Apoptosis. 2005;10:687–705.
2. Aggarwal BB, Danda D, Gupta S, Gehlot P. Models for prevention and treatment of cancer: Problems vs promises. Biochem Pharmacol. 2009;78:1083–1094.
3.Rang HP, Dale MM, Ritter JM, Flower RJ. In Rang and Dale's Pharmacology. 6 th ed. Churchill Livingston; 2007; p 718.
4.. Lishko VK, Lishko OV, Hoffman RM. The preparation of endotoxin-free L-Methionine-α-deamino-γ- mercaptomethane-lyase (L-Methioninase) from Pseudomonas putida. Protein expression and purification 1993; 4: 529-533.
5. Hoshiya Y, Inada T, Asanuma F, Yamada Y, Koh J, Kitojima M, Hoffman RM. Human tumors are methionine dependent in vivo. Anticancer research 1995; 15: 717-718.
6. Lishko VK, Lishko OV, Hoffman RM. Depletion of serum methionine by methioninase in mice. Anticancer Res 1993; 13: 1465-1468.
7. Kuldeep Kumar, Teena Phathak and Shefali Walia. L-arginase Based Biosensor for Detection of L-arginine in Juice Samples. J. Nat. Prod. Plant Resour ., 2012, 2 (4):494-499.
8..Markus Munder. Arginase: an emerging key player in the mammalian immune system. British Journal of Pharmacology (2009), 158, 638–651.
9. Kossel, A. and Dakin, H.D.(1904). Physiol. Chemie 41: 321-331.

10. Jenkinson CP, Grody WW, Cederbaum SD (1996). Comparative prop- erties of arginases. Comp Biochem Physiol B Biochem Mol Biol 114: 107–132.
11.Ryan J Viator**,** Richard F Rest**,** Ellen Hildebrandt and David J McGee Characterization of *Bacillus anthracis* arginase: effects of pH, temperature, and cell viability on metal preference. *BMC Biochemistry* 2008, 9:15.
12. Kuldeep Kumar, Teena Phathak and Shefali Walia. L-arginase Based Biosensor for Detection of L-arginine in Juice Samples. J. Nat. Prod. Plant Resour ., 2012, 2 (4):494-499.
13. Zellar, A., Van Orden, L.S. and Vogtili, A. (1954) Enzymology of mycobacteria.VII.
14. Prozesky, O.W., Grabron, W.O.K., Merwe, S.V., and Coetzee, J.N. (1973) Arginine cluster in Proteus Providence group. J. Gen. Microbiol., 77: 237-240.
15. Degryse, E.N., Glandroff, N. and Pierard, A. (1976) Arginine biosynthesis and degradation in an extreme thermophile strain Z05. Arch. Int. Physiol. Biochim., 84: 599-601.
16. Dessaux, Y.A., Petit, A., Tempe, J., Demarez, M., Legrain, C. and Wiame, J.M. (1976) Arginine catabolism in Agrobacterium strains: role of the Ti plasmid. J. Bacteriol., 166: 44-50.
17. Weathers, P.J., Chee, H.L. and Allen, M.M. (1978) Arginine catabolism in Aphanocapsa 6308. Arch Microbiol., 118(1): 1–6.
18. Gupta, M. and Carr, N.G. (1981) Enzymology of arginine metabolism in heterocyst forming cyanobacteria. FEMS Microb. Lett., 12: 179-181.
19. Simon, J.P. and Stalon, V. (1976) Purification and structure of arginase of Bacillus
20. Baumberg, S. and Harwood, C.R. (1979) Carbon and nitrogen repression of arginine catabolic enzymes in Bacillus subtilis. J. Bacteriol., 137: 189-196.
21. Vargha, G., Szabo, G. (1983) A conditional aerial mycelium mutant of Streptomyces fradiae with deficient ornithine cabamoyltransferase activity. J. Gen. Microbiol., 129: 539-542.
22. Bascaran, V., Haridsson, C. and Bran, A.F. (1989) Regulation of nitrogen catabolic enzymes in Streptomyces calvuligerus. J. Gen. Microbial., 135(9): 2465-2474.
23. Singh, S. (1994) Arginine metabolism in cyanobacterium Anabaena cycadeae: Regulation of arginine uptake and arginase by ammonia. Current Microbiology, 29(1): 49-52.
24. Patchett, M.L., Daniel, R.M. and Morgan, H.W. (1991) Characterization of arginase from the extreme thermophile 'Bacillus caldovelox'. Biochhim Biophys Acta., 1077(3): 291-298.
25. Moreno-Vivian, C., Soler, G. and Castillo, F. (1992) Arginine catabolism in the phototrophic bacterium Rhodobacter capsulatus E1FI . Purification and properties of arginase. Eur. J. Biochem., 204(2): 531-537.
26. Raines, K.W., Kang, T.J., Hibbs, S., Cao, G.L., Weaver, J., Tsai, P., Baillie, L., Cross, A.S. and Rosen, G.M. (2006) Importance of nitric oxide synthase in the control of infection by Bacillis anthracis. Infect Immun., 74(4): 2268-2276.
27. Hartenbach, S., Daoud-El Baba, M., Weber, W. and Fussenegger, M. (2007) An engineered L-arginine sensor of Chlamydia pneumoniae enables arginine- adjustable transcription control in mammalian cells and mice. Nucleic Acids Research, doi:10.1093/nar/gkm652: 1–13.
28. Yoshida, N. and Camargo, E.P. (1978) Ureotelism and ammonotelism in trypanosomatids. J. Bacteriol., 136(3): 1184-1186.
29. Elnekave, K., Siman-Tov, R. and Ankri, S. (2003) Consumption of L-arginine mediated by Entamoeba histolytica L-arginase(EhArg) inhibits amoebicidal activity and nitric oxide production by activated macrophages. Parasite Immunol., 25(11-12): 597-608.

30. Muller, I.B., Wrenger, C. (2005) Structural metal dependency of the arginase from the human malaria parasite Plasmodium falciparum. Biol Chem., 386(2): 117-126.
31.Borkovich, K. A. and Weiss, R.L. (1987) Relationship between two major Immunoreactive forms of Arginasse in Neurospora crassa. J Bacteriol., 169 (12): 5510-5517.
32. Dzikowska, A., Le Caer, J.P. , Jonczyk, P. and Weglenski, P. (1994) Purification of arginase from Aspergillus nidulans. Acta Biochim Pol., 41(4): 467-471.
33. El-Meleigy, M. A., Khattab, O. K. H. (1998) Partial purification and some characteristics of arginase of Trichoderma sp. Egyptian Journal of Microbiology, 33(1): 97-107.
34.Wagemaker, M.J., Welboren, W., Vander Drift., C., Jelten, M.S.,Griensven, L.J. and Camp Opalen, H.J. (2005) The ornithine cycle enzyme arginase from Agaricus bisporus and its role in urea accumulation in fruit bodies. Biochim Biophys Acta., 1681(2-3):107-115.
35. Planelles, V. (1987) Purification and some properties of the secreted arginase of the lichen Evernia prunastri and its regulation by usnic acid. Plant Science, 51(1): 9-16.
36. Legaz, M.E., Fontaniella, B., Millanes, A.M. and Vicente, C. (2004) Secreted arginases from phylogenetically far-related lichen species act as cross- recognition factors for two different algal cells. European Journal of Cell Biology, 83(8): 435-446.
37. Diez, A., Campo, M.L. and Soler, G. (1990) Properties of arginase immobilized in a fibrin clot. Biotechnol Appl Biochem., 12(3): 237-244.
38. Vivas, M., Sacristan, M., Legaz, M.E. and Vicente, C. (2009) The cell recognition model in chlorolichens involving a fungal lectin binding to an algal ligand can be extended to cyanolichens. Plant Biology., 12(4): 615-621.
39. Hensley, P. (1988) In Saccharomyces cerevisiae, ornithine transcarbamoylase and arginase form a regulatory multienzyme complex. Curr. Top. Cell. Regul., 29: 35- 45.
40. Kang, J.H. (1995) Purification and characterization of arginase from Schizosaccharomyces pombe. J. Biochem. Mol. Biol., 28(3): 232-237.
41. Cheema, P.S., Padmanaban, G. and Sharma, P.S. (1969) Arginase from Lathyrus sativus. Phytochemistry, 9: 404-411.
42. Kollofel, C. and Van-Duke, H.D. (1975) Mitochondrial arginase activity from cotyledon of developing and germinating seeds of Vicia faba L. Plant Physiol., 55: 507-510.
43. Roubelakis, K.A. and Kliewer, W.K. (1977) Enzymes of Krebs-Henseleit Cycle in Vitis-vinifera L. Plant Physiol., 62: 344-347.
44. Desai, H.V. (1983) Purification and properties of arginase from Arachis hypogea L.seedlings. Indian J. Biochem.Biophys., 20(4): 236-270.
45.Yu, G.H., Jun, B.O., Hong, Y.N. and Kwon, Y.M. (1988) Purification and characterization of Arginase from cotyledons of Canavalia lineata. Korean Biochem. J., 24(4): 497-504.
46. Kang, J.H. and Cho, Y.D. (1990) Purification and properties of arginase from Soybean, Glycine max, Axes. Plant Physiol., 93: 1230-1234.
47. Kavanaugh, D., Berge, M.A. and Rosenthal, G.A. (1990) A higher plant enzyme exhibiting broad acceptance of stereoisomers. Plant Physiol., 94: 67-70.
48. Hale, C.A., Clark, C.J., Petach, H.H. and Daniel, R.M. (1997) Arginase from kiwifruit:
49. Huang, L.W., Chang, K.L., Chen, C.J. and Liu, H.W. (2001) Arginase levels are increased in patients with rheumatoid arthritis. Kaohsiung J Med Sci.,. 17(7): 358-363.

50. Todd, C.D., Cooke, J.E., Mullen, R.T. and Gifford, D.J. (2001) Regulation of loblolly pine (Pinus taeda L.) arginase in developing seed tissue during germination and post-germinative growth. Plant Mol Biol., 45(5): 555-565.
51. Millanes, A.M., Fontaniella, B., Legaz, M.E. and Vincente, C. (2005) Glycoproteins from sugarcane plants regulate cell polarity Ustilago scitaminea teiospores. Journal of Plant Physiology, 162(3): 253-265.
52. Papadakis, A.K., Paschalidis, K.A., Roubelakis, A. and Angelakis, K.A. (2005) Biosynthesis profile and endogenous titers of polyamines differ in totipotent and recalcitrant plant protoplasts. Physiologia Plantarum, 125(1): 10-20.
53. Chen, H., McCaig, B.C., Howe, G.A. (2004) Regulation of plant arginase by wounding, jasmonate and the phytotoxin coronatine. J. Biol Chem., 279(44): 45998-46007.
54. Nabais, C., Hagemeyerb, J. and Freitasa, H. (2005) Nitrogen transport in the xylem sap of Quercus ilex: The role of ornithine. Journal of Plant Physiology., 162(5): 603-606.
55. Dabir, S., Dabir, P. and Somvanshi, B. (2005) Purification, properties and alternate substrate specificities of arginase from two different sources: Vigna catjang cotyledon and buffalo liver. Int J. Biol Sci., 1(3): 114-122.
56. Fahmy, A.S., (1994) Purification and characterization of arginase from eggs of the tick Hyalomma dromedarii. Egyptian Journal of Physiological Sciences, 18(1): 29-50.
57.Samson ML. (2000) Drosophila arginase is produced from a nonvital gene that contains the elav locus within its third intron. J Biol Chem., 275(40): 31107- 31114.
58. Tanuja D., Lazar K.V. and Mohamed U.V.K. (2005) Ontogenic changes in the levels of urea and arginase during the pupal to adult transformation in Bombyx mori. Journal of Entomological Research, 29(1): 61-63.
59. Santagata S. (2004) A waterborne behavioral cue for the actinotroch larva of Phoronis pallida (Phoronida) produced by Upogebia pugettensis (Decapoda: Thalassinidea). Biol Bull.., 207(2): 103-115.
60. Hiong, K.C., Loong, A.M., Chew, S.F. and Ip, Y.K. (2005) Increases in urea synthesis and the ornithine-urea cycle capacity in the giant African snail, Achatina fulica, during fasting or aestivation, or after the injection with ammonium chloride. J Exp Zool A Comp Exp Biol., 303(12): 1040-1053.
61. Loong, A.M., Hiong, K.C., Lee., S.M.L., Wong, W.P., Chew, S.F. and Ip, Y.K. (2005) Ornithine-urea cycle and urea synthesis in African lungfishes, Protopterus aethiopicus and Protopterus annectans, exposed to terrestrial conditions for six days. Journal of Experimental Zoology, 303A(5): 354-365.
62. Tesser, M.B., Terjesen, B.F., Zhang, Y., Portella, M.C. and Dabrowski, K. (2005) Free- and peptide-based dietary arginine supplementation for the South American fish pacu (Piaractus mesopotamicus). Aquaculture Nutrition, 11(6): 443-453.
63. Joerink, M., Savelkoul, H.F. and Wiegertjes, G.F. (2006) Evolutionary conservation of alternative activation of macrophages: structural and functional characterization of arginase 1 and 2 in carp (Cyprinus carpio L.). Mol Immunol., 43(8): 1116-1128.
64. Rodrigues, E., Ribeiro, A.C.M.T. and Bacila, M. (2006) L-Arginine metabolism in mitochondria isolated from the liver of antarctic fish Notothenia rossii and Notothenia neglecta. Brazilian Archives of Biology and Technology, 49(5): 825-833.

65. Kajimura, M., Walsh, P.J., Mommsen, T.P. and Wood, C.M. (2006) The dogfish shark (Squalus acanthias) increases both hepatic and extrahepatic ornithine urea cycle enzyme activities for nitrogen conservation after feeding. Physiol Biochem Zool., 79(3): 602-613.
66.Schindelmeiser, J., Schindelmeiser, I. and Greve, H. (1983) Hepatic arginase activity in intra- and extrauterine larvae of the ovoviviparous salamander, Salamandra salamandra (L.) (amphibia, urodela). Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 75(3): 471-473.
67. Carvajal, N., Ainol, L. (1987) Subcellular localization and kinetic properties of arginase from the liver of Genypterus maculatus. Comp Biochem Physiol B., 88(1): 229-231.
68. Prasad, G.V., Lokanatha, V., Sreekanth, K. and Rajender, W. (1997) Purification and kinetic properties of Mus booduga (Gray) hepatic arginase. J. Enzyme Inhib., 12(4): 255-272.
69. Steele, S.L., Yancey, P.H. and Wright, P.A. (2005) The little skate Raja erinacea exhibits an extrahepatic ornithine urea cycle in the muscle and modulates nitrogen metabolism during low-salinity challenge. Physiol and Biochem Zoology., 78(2): 216-226.
70. Aminlari, M. (1992) A novel colorimetric method for assaying arginase activity. Clinical Biochemistry, 25(6): 431-436.
71. Nikolaeva, S.D., Bakhtereva, V.T., Fok, E.M., Lavrova, E.A. and Parnova, R.G. (2008) Arginase activity in the frog urinary bladder epithelial cells and its involvement in regulation of nitric oxide production. Zh Evol Biokhim Fiziol., 44(3): 234-240.
72. Castaneda, M., Martuscelli, J. and Mora, J. (1967) The catabolism of L-arginine by Neurospora crassa. Biochimica et Biophysica Acta, 141(2): 276-286.
73. Mora, J.R., Sanchez, S. (1972) Regulation of arginase activity by intermediates of the arginine biosynthetic pathway in Neurospora crassa. J Bacteriol., 110: 870-877.
74. Chan, P.Y. and Cossins, E.A. (1973) Arginine metabolism in Saccharomyces cerevisiae. Some general properties of yeast arginase. Plant and Cell Physiology, 14(4): 641-651.
75. Weiss, R.L. (1976) Compartmentation and control of arginine metabolism in Neurospora. J Bacteriol., 126(3): 1173–1179.
76. Vaca, G. and Mora, J. (1977) Nitrogen regulation of Arginase in Neurospora crassa. J Bacteriology., 131(3): 719-725.
77. Broman, K., Lauwers, N., Stalon, V. and Wiame, J.M. (1978) Oxygen and nitrate utilization by Bacillus licheniformis of the arginase and arginine deiminase routes of arginine catabolism and other factors affecting their synthesis. J. Bacteriol., 134: 920-927.
78. Ellis, J.G., Kerr, A., Tempe, J. and Petit, A. (1979) Arginine catabolism: a new function of both octopine and nopaline Ti plasmid of Agrobacterium. Mol. Gen. Genet., 173: 263-269.
79. Rippka, R., Waterbury, J. B., Herdman, M. and Stanier, R. Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol., 111: 1-61.
80. Drainas, C and Weiss, R.L. (1980) Effect of carbon source on enzymes and metabolites of arginine metabolism in Neurospora, Journal of Bacteriology, 141(1): 205-212.
81. Schreier, H.J., Smith, T, M. and Bernlohr, R.W. (1982) Regulation of nitrogen catabolic enzymes in Bacillus spp. J Bacteriology., 151(2): 971-975.Science, 25: 295-301.
82. Marathe, S., Yu,Y.G., Turner,G.E., Palmier, C. and Weiss, R.L. (1998) Multiple forms of arginase are differentially expressed from a single locus in Neurospora crassa. J.Biol.Chem., 273(45): 29776-29785.

83.Breitburd, F.V. (1971) Rabbit liver L-arginase. J Biol Chem., 247(4): 1227-1235.
84. Grazi, E. and Magri, E. (1972) Molecular characteristics of chicken liver arginase. Biochem J., 126: 667-674.
85. Kaysan, G.A. and Strecker, H.J. (1973) purification and properties of arginase of rat kidney. Biochem J., 133: 779-788.
86. Tarrab, R., Rodriguez, J., Huitron, C., Palacios, R. and Soberon, G. (1974) Molecular forms of rat- liver arginase. Isolation and characterization. Eur J Biochem., 49: 457-468.
87. Reyero, C. and Dorner, F. (1975) Purification of arginases from human-leukemic lymphocytes and granulocytes: Study of their physicochemical and kinetic properties. European Journal of Biochemistry, 56(1): 137-147.
88. Fujimoto, M., Kameji, T., Kanaya, A. and Hagihira, H. (1976) Purification and properties of rat small intestinal arginase. J. Biochem., 79(2): 441-449.
89. Beruter, J., Colombo, J. and Bachmann, C. (1978) Purification and properties of arginase from human liver and erythrocytes. Biochem J., 175: 449-454.
90. Baranczyk-Kuzma, A., Zalejska, M. and Porembska, Z. (1980) Purification and some properties of human heart arginase. Acta Biochimica Polonica, 27(3-4): 181-189.
91. Gopalakrishna, R. and Agarrajan, B.N. (1980) Arginase from rat fibrosarcoma. Purification and properties. J Biosci., 2: 267-274.
92. Peiser, L. and Balinsky, J.B. (1982) Kinetic properties of arginase from Xenopus laevis. Comparative Biochemistry and Physiology, 73(2): 215-220.
93. Boutin, J.P. (1982) Purification, properties and subunit structure of arginase from Iris bulbs. Eur J Biochem., 127(2): 237-243.
94. Martin-Felequina,A. and Legaz, M.E. (1984) Purification and properties of the constitutive arginase of Evernia prunastri. Plant Physiol., 76(4): 1065-1069.
95. Kedra, M.L., Zamecka, E. (1988) The isolation and immunological properties of two arginase forms from human erythrocytes. Biochem Med Metab Biol., 39(3): 247-257.
96. Ikemoto, M., Tabata, M., Murachi, T. and Totani, M. (1989) Purification and properties of human erythrocyte arginase. Ann Clin Biochem., 26(6): 547-53.
97. Patil, N.B., Somvanshi, B.S. and Kothari, R.M. (1990) Isolation and characterization of arginase from Ox-erythrocyte. Biotech Tech., 4(2): 133-136.
98. Ikemoto, M., Tabata, M., Miyake, T., Kono, T., Mori, M., Totani, M. and Murachi, T. (1990) Expression of human liver arginase in Escherichia coli. Purification and properties of the product. Biochem J., 270(3): 697-703.
99. Singh, R.A. and Singh, S.N. (1990) Purification and properties of liver arginase from teleostean fish- Clarias batrachus(L). Arch. Int. Physiol. Biochim., 98(6): 411- 419.
100. Green S.M., Einstein, E., McPhiell, P. and Hensell, P. (1989) The purification and characterization of arginase from Saccharomyces cerevisiae. J Biol Chem., 265(3): 1601-1607.
101. Jenkinson, C.P. and Grigor, C.P. (1994) Rat mammary arginase: Isolation and characterization. Biochem Med Metab Biol., 51: 156–165.
102. Kuhn, N.J., Ward, S., Piponski, M. amd Young, T.W. (1995) Purification of human hepatic arginase and its manganese(II)- dependent and pH-dependent interconversion between active and inactive forms: a possible pH-sensing function of the enzyme on the ornithine cycle. Arch Biochem Biophys., 320(1): 24-34.

103. Mendz, G.L., Holwes, E.M. and Ferrero, R.L. (1998) In situ characterization of Helicobacter pylori arginase. Biochem Biophys Acta., 1388(2): 465-477.
104. Colleluori, D.M., Morris, S.M.Jr. and Ash, D.E. (2001) Expression, purification and characterization of human type II arginase. Arch Biochem Biophys., 389(1): 135-143.
105. Arakawa, N., Igarashi, M., Soda, K. (2003) D-arginase of Arthrobacter sp. KUJ 8602: Characterization and its identity with Zn2+ guanidinobutyrase. J Biochem., 133: 33-42.
106. McGee, D.J., Zabaleta, J., Viator, R.J., Testerman, T.L., Ochoa, A.C. and Mendz, G.L. (2004) Purification and characterization of Helicobacter pylori arginase RocF: unique features among the arginase superfamily. Eur J Biochem., 271: 1952- 1962.
107. Munder, M., F. Mollinedo, J. Calafat, J. Canchado, C. Gil-Lamaignere, J. M. Fuentes, C. Luckner, G. Doschko, G. Soler, K. Eichmann, F.-M. Müller, A. D. Ho, M. Goerner, and M. Modolell. (2005) Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood, 105: 2549-2556.
108. Mohamed, S.A., Fahmy, A.S., Mohamed, T.M. and Hamdy, S.M. (2005) Urea cycle of Fasciola gigantica: Purification andcharacterization of arginase. Comparative Biochemistry and Physiology, 142: 308 – 316.
109. Viator, R.J., Rest, R.F., Hildebrandt, E. and McGee, D.J. (2008) Characterization of Bacillus anthracis arginase: effects of pH, temperature, and cell viability on metal preference. BMC Biochemistry, 9(15): 1186-1471.
110. Reczkowski, R.S. and Ash, D.E. (1994) Rat liver arginase: kinetic mechanism, alternate substrates, and inhibitors. Archives of Biochemistry and Biophysics, 312(1): 31–37.
111. Kanyo, Z. F., Scolnick, L. R., Ash, D. E. and Christianson, D. W. (1996) Structure of a unique binuclear manganese cluster in arginase. Nature, 383: 554-557.
112. Bewley, M.C., Jeffrey, P.D., Baker, E.N. (1999) Crystal structures of Bacillus caldovelox arginase in complex with substrate and inhibitors reveal new insights into activation, inhibition and catalysis in the arginase superfamily. Structure, 7(4): 435-448.
113. Cox, J.D., Cama, E., Colleluori, D.M., Pethe, S., Boucher, J.L., Mansuy, D., Ash, D.E. Christianson, D.W. (2001) Mechanistic and metabolic inferences from the binding of substrate analogues and products to arginase. Biochemistry, 40: 2689–2701.
114. Cama, E., Colleluori, D.M., Emig. F.A., Shin, H., Kim, S.W., Kim, N.N., Traish, A.M., Ash, D.E. and Christianson, D.W. (2003). Human arginase II: crystal structure and physiological role in male and female sexual arousal. Biochemistry, 42(28): 8445–8451.
115. Di Costanzo, L., Sabio, G., Mora, A., Rodriguez, P.C., Ochoa, A.C., Centeno, F. and Christianson, D.W. (2005) Crystal structure of human arginase I at 1.29-Å resolution and exploration of inhibition in the immune response. PNAS 102(37): 13058-13063.
116. Colleluori, D.M., Cox, J.D., Scolnick, L.R., Compher, K., Jude, K., Han, S., Viola, R.E., Christianson, D.W. and Ash, D.E. (2005) Probing the role of the hyper-active histidine residue of arginase. Archives of Biochemistry and Biophysics, 444(1): 15-26.
117.Di Costanzo, L., Moulin, M., Haertlein, M., Meilleur, F. and Christianson, D.W. (2007). Expression, purification, assay, and crystal structure of perdeuterated human arginase I. Archives of Biochemistry and Biophysics, 465 (1): 82–89.
118. Dowling, D.P., (2010) Crystal Structure of arginase from Plasmodium falciparum and implications for L-arginine depletion in malarial infection. Biochemistry, 49 (26): 5600–5608.

119. Foster, L.B. and, J.M. (1971) A single-reagent manual method for directly determining urea nitrogen in serum. Clinical Chemistry, 17: 921-925.
120. Basch, J.J., Wickham, E.D.and Farrell, H.M. (1997) Arginase in lactating bovine mammary glands: implications in proline synthesis. J Dairy Sci., 80(12): 3241-3248.
121. Coulombe, J.J. and Favreau, L. (1963) A new simple semimicro method for colorimetric determination of Urea. Clinical Chemistry, 9: 102-108.
122. Davis, R.H. and Mora, J. J. (1968) Mutants of Neurospora crassa deficient in Ornithine-transaminase. J. Bacteriol., 96: 383-388.
123. Archibald, R.M. (1945) Colorimetric determination of urea. J. Biol Chem., 157: 507 518.
124. Aminlari, M. (1992) A novel colorimetric method for assaying arginase activity. Clinical Biochemistry, 25(6): 431-436.
125. Iyamu, E.W., Asakura, T. Woods, G.M. (2008) A colorimetric microplate assay method for high throughput analysis of arginase activity in vitro. Anal Biochem., 383(2): 332–334.
126. Booker, H.E. and Haslam, S.L. (1974) Immobilized enzyme electrode for the determination of arginase. Analytical chemistry, 46: 1054-1059.
127. Larsen, N.R., Hansen, E.H. and Guilbault, G.G. (1975) Enzyme analysis by means of the air-gap electrode-determination of urease and arginase by monitoring of the initial reaction rate. Anal. Chem. Acta., 79: 9-15.
128. Ward, R.L. and Srere, P.A. (1967) A new spectrophotometric arginase assay. Analytical Biochemistry, 18(1): 102-106.
129. Ozer, N. (1985) A new enzyme-coupled spectrophotometric method for the determination of arginase activity. Biochemical Medicine, 33(3): 367-371.
130. Han, S. and Viola, R.E. (2001) A spectrophotometric assay of arginase. Anal Biochem., 295(1): 117-119.
131. Orfanos, A.P., Guthrle, R. (1980) Fluorometric micromethod for determination of arginase activity in dried blood spots on filter paper. Clin Chem., 26(8): 1198-1200.
132. Macholan, L., Filipck, K. and Andrys. C. (1989) Assays of urea and arginase with a biocatalytic membrane electrode operating on conductimetric principle. Willy Interscience: Journal : Abstract.
133. Carulli, N., Kaihara, S. and Wagner, H.N. Jr. (1967) Radioisotopic assay of arginase activity. Analytical Biochemistry, 24(3): 515-522.
134. Volke, A., Wegener, G., Vasar, E. and Volke, V. (2006) High-performance liquid chromatography method with radiochemical detection for measurement of nitric oxide synthase, arginase, and arginine decarboxylase activities. Methods and findings in Experimental and Clinical Pharmacology, 28(1): 3-6.
135. de Bono, J.P., Warrick, N., Bendall, J.K., Channon, K.M. and Alp, N.J. (2007) Radiochemical HPLC detection of arginine metabolism: measurement of nitric oxide synthesis and arginase activity in vascular tissue. Nitric Oxide, 16(1): 1- 9.
136. Bastone, A., Diomede, L., Parini, R., Carnevale, F. and Salmona, M. (1990) Determination of argininosuccinate lyase and arginase activities with an amino acid analyzer. Analytical Biochemistry, 191(2): 384-389.
137. Wheatley, D.N., Philip, R. and Campbell, E. (2003) Arginine deprivation and tumor cell death: Arginase and its inhibition. Molecular and Cellular Biochemistry, 244: 177-185

138.Cheng, N.M., Lo, W.H. (2005) Pharmaceutical preparation and method of treatment of human malignancies with arginine deprivation. US Patent Application No. 20050244398.
139. Cheng, N.M. (2006) Patent application title: Pharmaceutical Composition and Method of Treating Hepatitis with Arginases. International Application No.:PCT/CN2005/001411.
140. Cheng, N.M., Leung, Y.C. and Lo, W.H. (2006) Use of arginase in combination with 5FU and other compounds for treatment of human malignancies. International Application No. PCT/CN2005/002001.
141. Hsueh, E.C., Knebel, S., Collier, I., Kadze, M., Hsueh, C., Lo, T., Cheng, P. and Leung, T. (2006) Recombinant arginase as a novel anti-melanoma agent. Journal of Clinical Oncology, 24(18S): 12032.
142. Cheng, P.N.M., Lam, T.L., Lam, W.M., Tsui, S.M., Cheng, A.W.M.., Lo, W.H. and Leung, Y.C. (2007) Pegylated recombinant human arginase (rhArg- peg5,000mw) inhibits the in vitro and in vivo proliferation of human hepatocellular carcinoma through arginine depletion. Cancer Res., 67: 309- 317.
143. Tsui, S.M., Lam, W.M., Lam, T.L., Chong, H.C., So, P.K., Kwok, S.Y., Arnold, S., Cheng, P.N.M, Wheatley, D.N., Lo, W.H. and Leung, Y.C. (2009) Pegylated derivatives of recombinant human arginase (rhArg1) for sustained in vivo activity in cancer therapy: preparation, characterization and analysis of their pharmacodynamics in vivo and in vitro and action upon hepatocellular carcinoma cell (HCC). Cancer Cell International, 9:9 doi:10.1186/1475-2867- 9-9.
144. Lam, T.L., Wong, G.K., Chong, H.C., Cheng, P.N., Choi, S.C., Chow, T.L., Kwok, S.Y., Poon, R.T., Wheatley, D.N., Lo, W.H. and Leung, Y.C. (2009) Recombinant human arginase inhibits proliferation of human hepatocellular carcinoma by inducing cell cycle arrest. Cancer Lett., 277(1): 91-100.
145. Stone, E.M., Glazer, E.S., Chantranupong, L., Cherukuri, P., Breece, R.M., Tierney, D.L., Curley, S.A., Iverson, B.L. and Georgiou, G. (2010) Replacing Mn2+ with Co2+ in human arginase I enhances cytotoxicity toward L-arginine auxotrophic cancer cell lines. ACS Chem. Biol., 5 (3): 333–342.
146. Hernandez, C.P., Morrow, K., Lopez-Barcons, L.A., Zabaleta, J., Sierra, R., Velasco, C., Cole, J. and Rodriguez, P.C. (2010) Pegylated arginase I: a potential therapeutic approach in T-ALL. Blood, 115(25): 5214-5221.
147. Haraguchi, Y., Takiguchi, M., Amaya, Y., Kawamoto, S., Matsuda, I. and Mori, M. (1987) Molecular cloning and nucleotide sequence of c-DNA for human liver arginase. Proc Natl Acad Sci USA., 84(2): 412-415.
148. Maria, C.B., Lett,J.S., Baker,E.N., and Patchett, M.L. (1996) The cloning, expression and crystallization of a thermostable arginase. FEBS Letters, 386: 215-218.
149. Shimotohno, K.W., Miwa, I. and Endo, T. (1997) Molecular cloning and nucleotide sequence of the arginase gene of Bacillus brevis TT02-08 and its expression in Escherichia coli. Biosci Biotechnol Biochem., 61(9): 1459-1464.
150. Tuchman, M., Rajagopal, B.S., McCann, M.T. and Malamy, M.H. (1997) Enhanced production of arginine and urea by genetically engineered Escherichia coli K- 12 strains. Appl. Environ. Microbiol., 63(1): 33-38.

151. Salimuddin, Upadhyaya, K.C., Raju, J. and Baquer, N.Z. (1999) Modulation of m-RNA levels of liver arginase by insulin and vanadate in experimental diabetics. Indian J. Biochem Biophys., 36(2): 125-128.
152. Kimura, M., Tatsumi, K., Tada, H., Ikemoto, M., Fukuda, Y., Kaneko, A., Kato, M., Hidaka, Y. and Amino, N. (2000) Enzyme immunoassay for autobodies to human liver-type arginase and its clinical applications. Clinical Chemistry, 46: 112-117.
153. da Silva, E.R., da Silva, M.F., Fischer, H., Mortara, R.A., Mayer, M.G., Framesqui, K., Silber, A.M. and Floeter-Winter, L.M. (2008) Biochemical and biophysical properties of a highly active recombinant arginase from Leishmania (Leishmania) amazonensis and subcellular localization of native enzyme. Mol Biochem Parasitol., 159(2): 104-111.
154. Carvajal, N., Martinez, J. and Fernandez, M. (1977) Immobilised monomers of human liver arginase. Biochim Biophys Acta., 481(1): 177-183.
155. Muszynska, G. and Wojtczak, M. (1979) Influence of immobilization on conformation of rat liver arginase. International Journal of Biochemistry, 10(8): 665-668.
156. Rossi, V., Malinverni, A. and Callegaro, L. (1981) Immobilization of arginase on hollow fiber hemodialyzer. Int J Artif Organs., 4(2):102-107.
157. Aguirre, R. and Kasche, V. (1983) Catalytically active monomer forms of immobilized arginase. Eur J Biochem., 130: 373-381.
158. Savoca, K.V., (1984) Cancer therapy with chemically modified enzyme: II . The effectiveness of arginase modified by the covalent attachment of polyethylene glycol, on a Taper liver tumor and the L51784 murine leukemia. Cancer Biochem Biophys., 7: 261-268.
159. Visco, C., Benassi, C.A., Veronese, F.M. and Miglioli, P.A. (1987) Purification, modification, physico-chemical and pharmacokinetic characterization of arginase-an enzyme of potential use in therapy. Farmaco Sci. J., 42(8): 549- 559.
160. Veronese, F.M., Lora, S., Carenza, M. and Palma, G. (1988) Properties and potential applications of arginase immobilized by radiation- induced polymerization of acrylic monomers. Annals of the New York Academy of Sciences, 542(9): 115-120.
161. Daghigh, F., Ash D.E. (1992) Chemical modification and inactivation of rat liver arginase by N- bromosuccinimide: reaction with His 141. J Bacterial., 174(1): 48-55.
162. Dala, E. and Szajani, B. (1994) Immoblization, characterization and laboratory-scale application of bovine liver arginase. Appl. Biochem Biotechnol., 49(3): 203-215.
163. Alonso, A., Almendral, M.J., Baez, M.D., Porras, M.J. and Alonso, C. (1995) Enzyme immobilization on an epoxy matrix. Determination of L-arginine by flow- injection techniques. Analytica Chimica Acta, 308(1-3): 164-169.
164. Bagnost, T., Robert, J.F., Berthelot, A., Xicluna, A. and Andre, C. (2007) Immobilization of arginase and its application in an enzymatic chromatographic column: thermodynamic studies of nor- NOHA/arginase binding and role of the reactive histidine residue. J Chromatogr B Analyt Technol Biomed Life Sci., 856(1-2): 113-120.
165. Silva, E.R. and Floeter-Winter, L.M. (2010) Activation of Leishmania (Leishmania) amazonensis arginase at low temperature by binuclear Mn2+ center formation of the immobilized enzyme on a Ni2+ resin. Experimental Parasitology, 125(2): 152-155.

166. Konst, P.M., Turras, P.M.C.C.D., Franssen, M.C.R., Scott, E.L. and Sanders, J.P.M. (2010) Stabilized and immobilized Bacillus subtilis arginase for the biobased production of nitrogen-containing chemicals. Advanced Synthesis & Catalysis, 352(9): 1493-1502.
167. Puri, D. and Kaul, N. (1995) Further studies on serum arginase as indicator of hepatocellular damage. Indian Journal of Clinical Biochemistry, 10(1): 42-44.
168. Esch, F., Lin, K.I., Hills, A., Zaman, K., Baraban, J.M., Chatterjee, S., Rubin, L., Ash, D.E. and Ratan, R.R. (1998) Purification of a multipotent antideath activity from bovine liver and its identification as arginase: nitric oxide-independent inhibition of neuronal apoptosis. J Neurosci., 18(11): 4083-4095.
169. Corraliza, I. and Moncada, S. (2002) Increased expression of arginase II in patients with different forms of arthritis. Implications of the regulation of nitric oxide. The Journal of Rheumatology, 29(11): 2261-2265.
170. Mori, M. and Gotoh, T. (2004) Arginine Metabolic Enzymes, Nitric Oxide and Infection. J. Nutr., 134(10): 2820S-2825S.
171. Kim, N.N., Christianson, D.W. and Traish, A.M. (2004) Role of arginase in the male and female sexual arousal response. Journal of Nutrition, 134(10): 2873S- 2879S.
172. Lange, P.S., Langley, B., Lu, P. and Ratan, R.R. (2004) Novel roles of arginase in cell survival, regeneration, and translation in the central nervous system. Journal of Nutrition, 134(10): 2812S-2817S.
173. Munder, M., F. Mollinedo, J. Calafat, J. Canchado, C. Gil-Lamaignere, J. M. Fuentes, C. Luckner, G. Doschko, G. Soler, K. Eichmann, F.-M. Müller, A. D. Ho, M. Goerner, and M. Modolell. (2005) Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood, 105: 2549-2556.
174. Scaglia, F. and Lee, B. (2006) Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. Am J Med Genet C Semin Med Genet., 142C(2): 113-20.
175. Mielczarek, M., Chrzanowska, A., Scibior, D., Skwarek, A., Ashamiss, F., Lewandowska, K. and Baranczyk-Kuzma, A. (2006) Arginase as a useful factor for the diagnosis of colorectal cancer liver metastases. Int J Biol Markers., 21(1): 40-44.
176. Munder, M., Schneider, H., Luckner, C., Giese, T., Langhans, C.D., Fuentes, J.M., Kropf, P., Mueller, I., Kolb, A., Modolell, M. and Ho, A.D. (2006) Suppression of T-cell functions by human granulocyte arginase. Blood, 108(5): 1627-1634.
177. Eskiocak, S., Gozen, A.S., Taskiran, A., Kilic, A.S., Eskiocak, M. and Gulen, S. (2006) Effect of psychological stress on the L-arginine-nitric oxide pathway and semen quality. Braz J Med Biol Res., 39(5): 581-588.
178. Cao, W., Wu, T., Tur-Kaspa, R. and Fan, Q. (2009) Hepatitis C virus targets over-expression of arginase I in hepatocarcinogenesis. Int J Cancer., 124(12): 2886-2892.
179. King, N.E., Rothenberg, M.E. and Zimmermann, N. (2004) Arginine in asthma and lung inflammation. Journal of Nutrition, 134(10): 2830S-2836S.
180. Morris, C.R., Poljakovic, M., Lavrisha, L., Machado, L., Kuypers, F.A. and Morris, S.M. Jr. (2004) Decreased Arginine Bioavailability and Increased Serum Arginase Activity in Asthma. American Journal of Respiratory and Critical Care Medicine, 170: 148-153.

181. Morris C.R., Kato, G.J., Poljakovic, M., Wang, X., Blackwelder, W.C., Sachdev, V., Hazen, S.L., Vichinsky, E.P., Morris, S.M. Jr. and Gladwin, M.T. (2005) Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. Journal of the American Medical Association, 294(1): 81-90.
182. Grasemann, H., Schwiertz, R., Matthiesen, S., Racke, K. and Ratjen, F. (2005) Increased Arginase Activity in Cystic Fibrosis Airways. American Journal of Respiratory and Critical Care Medicine, 172: 1523-1528.
183. Schnorr, O., Schuier, M., Kagemann, G., Wolf, R., Walz, M., Ruzicka, T., Mayatepek, E., Laryea, M., Suschek, C.V., Kolb-Bachofen, V. and Sies, H. (2005) Arginase-1 overexpression induces cationic amino acid transporter-1 in psoriasis. Free Radic Biol Med., 38(8): 1073-1090.
184. Steppan, J., Ryoo, S., Schuleri, K.H., Gregg, C., Hasan, R.K., White, A.R., Bugaj, L.J., Khan, M., Santhanam, L., Nyhan, D., Shoukas, A.A., Hare, J.M. and Berkowitz, D.E. (2006) Arginase modulates myocardial contractility by a nitric oxide synthase 1-dependent mechanism. Proc Natl Acad Sci USA., 103(12): 4759–4764.
185. Bjelakovic, G., Sokolovic, D., Ljiljana, S., Kocic, G., Jevtovic, T., Stojanovic, I., Ilic, M., Bjelakovic, L., Nikolic, J. and Basic, J. (2009) Arginase activity and magnesium levels in blood of children with diabetes mellitus. J Basic Clin Physiol Pharmacol., 20(4): 319-334.
186. Munder, M. (2009) Arginase: an emerging key player in the mammalian immune system. British Journal of Pharmacology, 158(3): 638 - 651.
187. Maarsingh, H., Meurs, H. (2009) Arginase: a key enzyme in the pathophysiology of allergic asthma opening novel therapeutic perspectives. Br J Pharmacol., 158(3): 652-664.
188. Munder, M. (2010) Role of arginase in asthma: potential clinical applications. Expert Review of Clinical Pharmacology, 3(1): 17-23.