General introduction and outline of the thesis

HUMAN IMMUNODEFICIENCY VIRUS

In 1983 the Human immunodeficiency virus (HIV) was discovered after the clinical observation in 1981 of a cluster of patients who were suffering from *Pneumocystis jirovecii* (previously carinii) pneumonia.[1] This rare opportunistic infection was only reported in patients with a severely impaired immune system, but this cluster of patients did not have a medical history of immune deficiency. In 2017, there were an estimated 36.9 million (31.1-43.9 million) people living with HIV worldwide and in July 2018 21.7 million (19.1-22.6 million) of those people had access to antiretroviral therapy.[2]

The HIV is a single-stranded ribonucleic acid (RNA) virus, that specifically infects $CD4^+$ T-helper lymphocytes, macrophages and dendritic cells by binding of glycoprotein 120, a protein on its trimeric envelope complex, to the CD4-receptor and one of the co-receptors CCR5 or CXCR4 on the target cell.[3-5] After a series of steps, HIV glycoprotein 41 is inserted into the host cellular membrane and then undergoes a significant conformational change, forming a hairpin and bringing the membranes of the HIV and the host cell together, thereby allowing them to fuse.[6] After the fusion process, the HIV capsid enters the cytoplasm of the target cell, where it releases its content: the enzymes reverse transcriptase, integrase and protease, some minor proteins, the major core protein and two single-stranded RNA strands.[7] Inside the cytoplasm, HIV uses its enzyme reverse transcriptase and the nucleotides of the host to transform its single-stranded RNA into a double-stranded deoxyribonucleic acid (DNA) molecule.[8] This DNA is transported to the nucleus of the host cell and via the enzyme integrase incorporated into the DNA of the host.[9] The nucleotide metabolism of the host further transcribes this incorporated DNA into new RNA strands, that, after translation of new essential HIV proteins, are assembled together with these proteins into a new HIV virion that buds from the host membrane and is released from the host cell.[7] Finally, the HIV enzyme protease completes the last step of the HIV cycle, maturing the virus to a new infectious particle. The HIV-cycle is displayed in Figure 1.

If left untreated, HIV infection eventually leads to a depletion of $CD4^+$ T-lymphocytes. The mechanism behind the slow decrease of $CD4^+$ T-lymphocytes is not completely understood, but probably consists of a combination of factors. HIV is causing a direct cytotoxicity, leading to massive $CD4^+$ T-cell destruction, occurring in the early course of the infection. It is thought that this massive destruction is followed by a regenerative response of $CD4^+$ memory T-cells, preserving $CD4^+$ T-cell numbers and immune function. However, this homeostatic balance eventually fails, due to factors such as infection and death of progenitor cells,[10] destruction of the secondary lymphoid tissues that support the homeostasis[11] and chronic inflammation. The chronic inflammation is also sustained by a combination of factors, like gastrointestinal mucosa loss (leading to microbial translocation),[12] pyroptosis (a

highly inflammatory form of programmed cell death like apoptosis, leading to the release of the dying cells contents among which inflammatory cytokines)[13] and polyclonal activation of B-cells[14] and pro-inflammatory cytokines.[15] Failing of the homeostasic balance between $CD4^+$ T-cell destruction and regeneration, leads to a critical effector T-cell population loss,[16] resulting in the Acquired Immune Deficiency Syndrome (AIDS). AIDS is the last stage of HIV-infection, defined by a $CD4^+$ T-cell count below 200 cells/mm^3 or the presence of an AIDS-defining illness, which are opportunistic infections or certain types of cancer, and ultimately death. However, this lethal chain of events can be reversed by combination antiretroviral therapy; cART. Antiretroviral therapy has become increasingly effective over the last decades in inhibiting the replication of HIV and enabling the restoration of the immune system. The drugs used in antiretroviral therapy for HIV can be categorized as CCR5 antagonists, fusion and entry inhibitors, non-nucleoside reverse transcriptase inhibitors (NNRTI), reverse transcriptase inhibitors that are nucleoside/nucleotide analogues (NRTI), integrase inhibitors (INSTI) and protease inhibitors (PI), based on their target in the HIV replication cycle (Figure 1). A cART regimen consists of a combination of drugs from different classes. According to all commonly used guidelines[17-21] the preferred initial treatment for all cART naïve patients consists of two NRTIs combined with either an NNRTI, a PI with a pharmacoenhancer (also called a booster) or an integrase inhibitor. Very recent studies, however, have shown equal effectiveness with combinations of 2 drugs instead of 3, and thus the guidelines might be adapted on short notice.[22-24]

After the introduction of cART, the life-expectancy of people living with HIV is now approaching the life-expectancy of the non-HIV infected general population in the Western world.[25] Additionally, cART significantly reduces new HIV transmissions to HIV-negative sexual partners either by treatment of the HIV-positive partner (Treatment as prevention; TASP)[26,27] or by treating the HIV-negative partner prior to sexual intercourse (Pre-exposure prophylaxis; PREP).[28] Although these therapeutic strategies are highly effective, cART is not curative and cessation leads to a rebound of HIV viremia. Treatment of HIV requires a lifelong commitment to medication with the risk of adverse effects, sometimes severe, which have been reported for all drugs used in cART regimens. Besides HLA-B*57:01, a marker to predict hypersensitivity for abacavir, no other biomarkers or genetic susceptibility traits are known to predict the occurrence of adverse events during cART use.

Thus HIV treatment needs to be further ameliorated, in order to diminish adverse reactions during cART use, and new biomarkers to predict adverse reactions would be very helpful tools. Both the replication of HIV and the mechanism of action of the NRTIs, the backbone of the cART regimens currently recommended in the HIV treatment guidelines, depend for an important part on the human nucleotide metabolism. Further, also in other viral infections, like hepatitis C (HCV), hepatitis B (HBV) and herpesviridae, nucleotide analogues

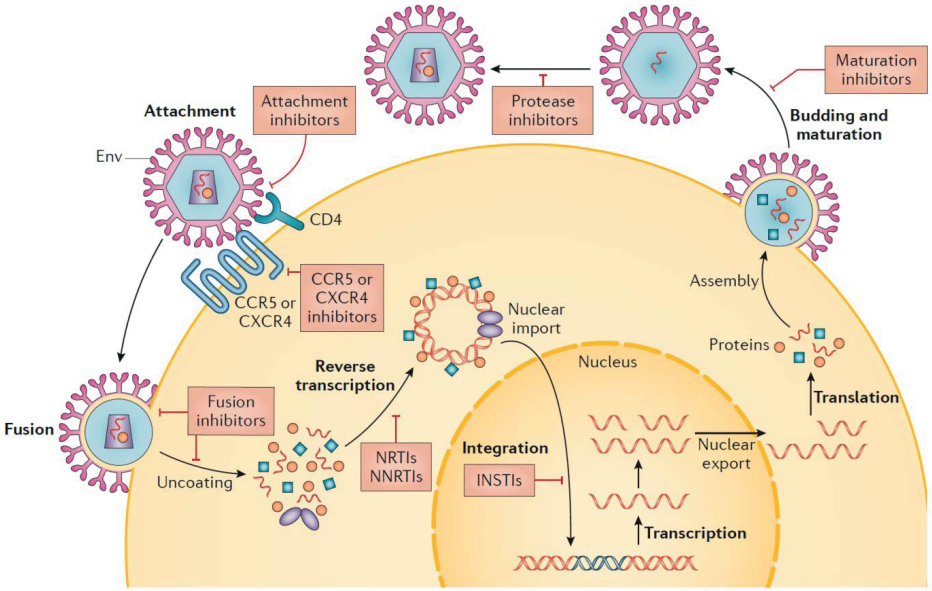

Figure 1. HIV replication cycle and drug targets for combination antiretroviral therapy. *Adapted from: Deeks, S.G.; Overbaugh, J.; Phillips, A.; Buchbinder, S.; HIV Infection. Nature Reviews Disease Primers, October 2015; Vol 1; p1-22. Licensed by Springer Nature RightsLink.*

are used in the treatment. Knowledge regarding the nucleotide metabolism is therefore important to improve inhibition of HIV replication and decrease adverse events during therapy with nucleotide analogue drugs in HIV and various other infectious diseases.

HUMAN NUCLEOTIDE METABOLISM

Canonical nucleotides

Nucleotides are the building blocks of DNA and RNA, and the basis for the function of all cells in all living species (including humans). In humans, DNA is located in the nucleus (chromosomal DNA) and in the mitochondrion (circular mtDNA). Nucleotides contain a nucleobase, a deoxyribose (together a nucleoside) and a phosphate group (Figure 2a). In RNA the nucleoside contains a ribose instead of a deoxyribose.

DNA is composed of 2 strings of nucleotides, bound together via the nucleobases by hydrogen bonds and forming the genetic code (Figure 2b). The nucleobases incorporated in nucleic acids are adenine, guanine, cytosine and thymine, and are considered the canonical nucleobases. These nucleobases can be divided in 2 groups according to their chemical structure. Cytosine and thymidine (and uracil in RNA) are pyrimidines and have a core in

the shape of a pyrimidine ring (C4H4N2). Adenine and guanine are purines and have a core consisting of a heterocyclic aromatic ring (with the same structure as the pyrimidine ring) fused to a imidazole ring (C3H4N2) (Figure 3).

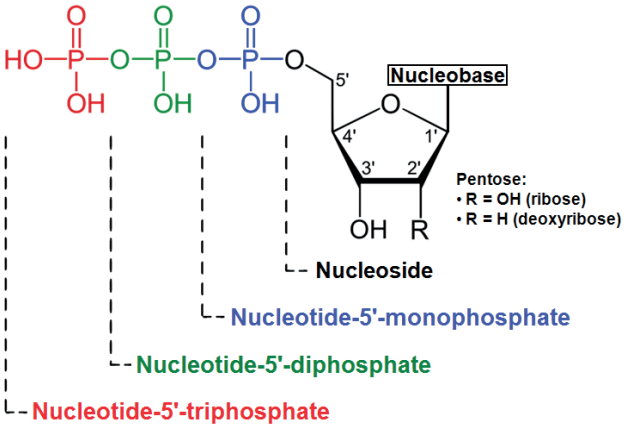

Figure 2a. Chemical structure of nucleoside and nucleotide 5'-mono-, di- and triphosphate. *Adapted from: Yikrazuul, own work, general overview of nucleotides and nucleosides, may 26th 2010, URL https://commons.wikimedia.org/wiki/File:Nucleotide_nucleoside_general.svg, accessed June 28th 2018.*

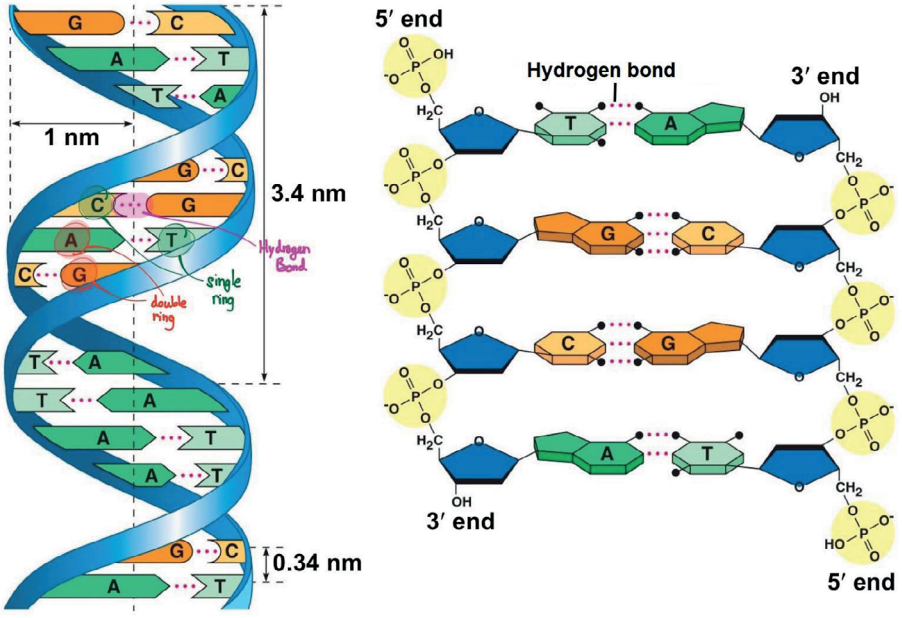

Figure 2b. Schematic DNA structure (left) and nucleobase bonds (right). *Adapted from: Pearson Education, Inc., publishing as Pearson Benjamin Cummings, URL http://logyofbio.blogspot.com/2016/02/structure-of-dna-and-nucleotides.html, accessed June 28th 2018.*

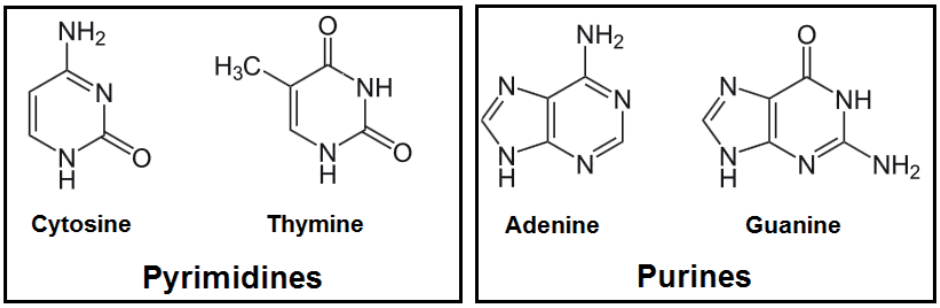

Figure 3. Chemical structure of the canonical pyrimidines and purines.

Role of canonical nucleotides in human metabolism

Besides being building blocks for DNA, the nucleotides have additional important functions in the human cell metabolism. The nucleotide-5'-triphosphates function as energy carriers because energy is released when the phosphate groups are hydrolysed from the nucleoside base by kinases. From the nucleotide-5'-triphosphates, adenosine 5'-triphosphate (ATP) is the most preferred nucleotide for most cellular processes. The preference for ATP has been observed in a large study investigating over 200 kinases and found that most had affinity for ATP and only a small number of the kinases exhibited affinity for guanosine 5'-triphosphate (GTP).[29] Further, cyclic nucleotides act as secondary messengers, transducing signals from outside the cells to intracellular. For example: the primary messenger epinephrine stimulates the liver cell via triggering the secondary messenger cyclic adenosine 3'5'-monophosphate (cAMP) to convert glycogen to glucose (glycogenolysis).[30] cAMP is generated from ATP by adenylyl cyclase and has three major targets in most cells: protein kinase A (PKA), exchange proteins activated by cAMP (Epacs) and cyclic-nucleotide-gated ion channels.[31] PKA phosphorylates numerous metabolic enzymes, among which enzymes regulating glycogen, sugar and lipid metabolism, depending on the cell type. The Epac proteins are involved in multiple cellular functions such as (among others) cell adhesion, cell differentiation, apoptosis and secretion.[32-34] Cyclic guanine 3'5'-monophosphate (cGMP), produced from GTP by two families of guanylyl cyclases: transmembrane particulate guanylyl cyclase (pGC) and soluble guanylyl cylclase (sGC), activate protein kinase G (PKG). PKG phosphorylates several enzymes responsible for multiple cellular processes, like vascular tone and remodeling, neuronal adaptation, intestinal water secretion and bone growth.[35] cGMP modulates cAMP concentration[36] and, like cAMP, also regulates cyclic nucleotide-gated ion channels.[37] Nucleotides are also important constituents of the coenzymes nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$) and Flavin adenine dinucleotide (FAD), which play a role in oxidation-reduction reactions. And finally, nucleosides are involved in the synthesis of polysaccharides, for instance UDP-glucose which is an intermediate in the glycogen synthesis in mammals.[38]

Purine nucleotide synthesis

In the human metabolism two pathways of purine nucleotide synthesis exist: the 'de novo' synthesis pathway, in which the purine base is synthesized step by step on the ribose-5'-phosphate, and the salvage pathway, in which a ribose-5'-phophate is added to the preformed purine base or phosphates are added to a preformed, or rather recycled, purine nucleobase. In the de novo purine synthesis pathway (Figure 4), inosine 5'-monophosphate (IMP) is formed from the active form of ribose: 5-phosphoribosyl-1-pyrophosphate (PRPP) through 10 enzymatic steps. IMP is further converted into either adenosine 5'-monophosphate (AMP) or guanosine 5'-monophosphate (GMP) in two final steps (Figure 5). To form AMP, first the carbonyl oxygen atom at C6 is substituted for an amino group on which aspartate is added by the enzyme adenylosuccinate synthetase. Finally, fumarate is released from the amino group by adenylosuccinase. GMP is formed by oxidation of IMP at C2 by IMP dehydrogenase using NAD^+ and H2O, followed by transfer of the amido-N of glutamine to the C2 position by GMP synthetase. Note that for the synthesis of AMP, GTP is used and for the synthesis of GMP, ATP is used.

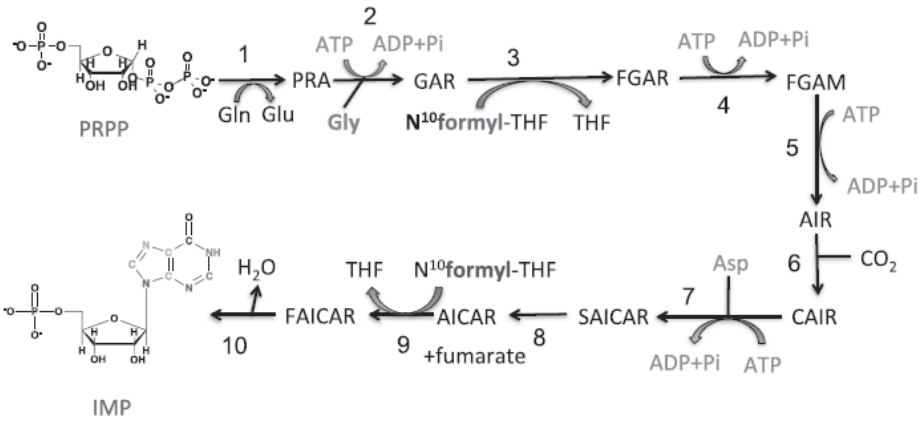

Figure 4. Purine nucleotide de novo pathway. Inosine 5'-monophosphate (IMP) is formed from 5-phosphoribosyl-1-pyrophosphate (PRPP) in 10 enzymatic steps: (1) glutamine phosphoribosylpyrophosphate amidotransferase (GPAT activity - *PPAT* gene); (2) glycinamide ribonucleotide synthetase (GARS activity - *GART* gene); (3) glycinamide ribonucleotide formyltransferase (GART activity - *GART* gene); (4) phosphoribosylformylglycinamide synthase (PFAS activity - *PFAS* gene); (5) aminoimidazole ribonucleotide synthetase (AIRS activity - *GART* gene); (6) aminoimidazole ribotide carboxylase (AIRC activity - *PAICS* gene); (7) succinylaminoimidazolecarboxamide ribonucleotide synthetase (SAICAR activity - *PAICS* gene); (8) adenylosuccinate lyase (ADSL activity - *ADSL* gene); (9) 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase (AICARFT activity - *ATIC* gene); (10) IMP cyclohydrolase (IMPCH activity - *ATIC* gene). *From: Lane A.N. and Fan T.W.-M. Regulation of mammalian nucleotide metabolism and biosynthesis. Nucleic Acids Research, 2015, Vol 43(4): p.2466-2485.*

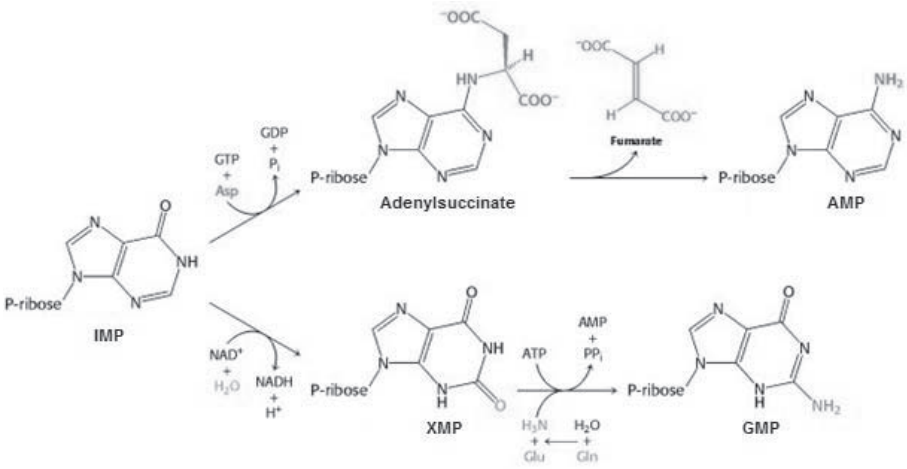

Figure 5. Interconversion of purines; synthesis of AMP and GMP from IMP. *Adapted from: Berg J.M., Tymoczko J.L. and Stryer L. Biochemistry. 5th edition 2002. New York: W.H. Freeman.*

In erythrocytes the 'de novo' pathway is absent, so the erythrocyte relies completely on the salvage pathway (Figure 6) for the requirement of the purine nucleotides. The salvage pathway provides a way to utilize purine bases derived from diet (exogenous) or from the normal turnover of nucleic acids (endogenous) and reconverts these purine bases to their corresponding nucleotides by phosphoribosylation. As in the de novo synthesis pathway, PRPP serves as the activated ribose-5-phosphate. The enzymes involved in the process of resynthesis of nucleotides from bases are adenine phosphoribosyltransferase (APRT) for adenine and hypoxanthine-guanine phophoribosyltransferase (HGPRT) for hypoxanthine and guanine. Thereafter, the purine nucleosides are degraded in several steps to the ultimate end product uric acid, which is excreted in the urine.

NRTIs in HIV

The mechanism of action of the NRTIs, used as backbone of the cART regimens currently recommended in HIV treatment guidelines, is based on the human nucleotide metabolism. Abacavir, tenofovir disoproxil fumarate (from now on referred to as tenofovir) and didanosine (which is currently no longer widely used) are NRTIs resembling the natural purine nucleosides (Figure 7); abacavir and didanosine being guanosine nucleoside analogues and tenofovir being an adenine nucleotide analogue.

These drugs are first metabolized inside the cells and converted to their active 5'-triphosphate forms. For instance, carbovir is the active 'triphosphate' form of abacavir. The active 5'-triphosphate forms compete with the natural purine nucleotide triphosphates for incorporation in the growing viral DNA, but due to the lack of a 3'-hydroxyl group, the next nucleotide cannot be added to the DNA strand and further DNA synthesis is terminated.

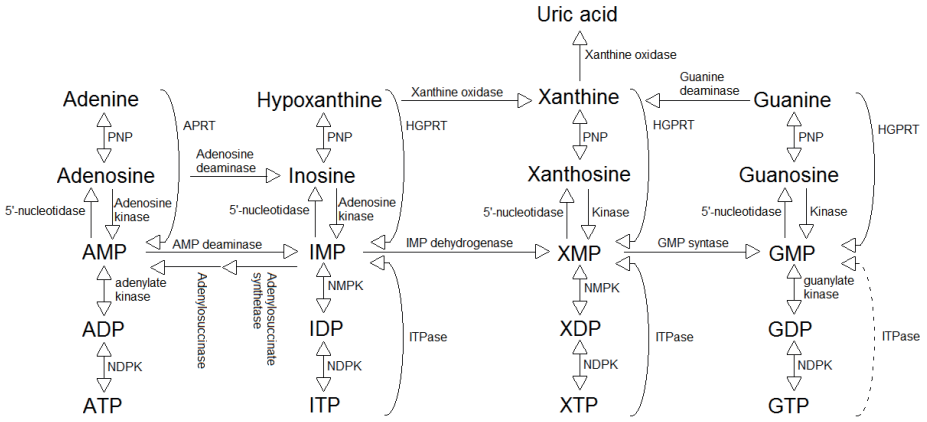

Figure 6. Purine salvage pathway. PNP, Purine nucleotide phosphorylase; APRT, Adenine phosphoribosyltransferase; HGPRT, Hypoxanthine-guanine phosphoribosyltransferase; AMP, Adenosine 5'-monophosphate; IMP, Inosine 5'-monophosphate; XMP, Xanthosine 5'-monophosphate; GMP, Guanosine 5'-monophosphate; NMPK, Nucleoside monophosphate kinase; ADP, Adenosine 5'-diphosphate; IDP, Inosine 5'-diphosphate; XDP, Xanthosine 5'-diphosphate; GDP, Guanosine 5'-diphosphate; NDPK, Nucleoside diphosphate kinase; ATP, Adenosine 5'-triphosphate; ITP, Inosine 5'-triphosphate; XTP, Xanthosine 5'-triphosphate; GTP, Guanosine 5'-triphospposphate.

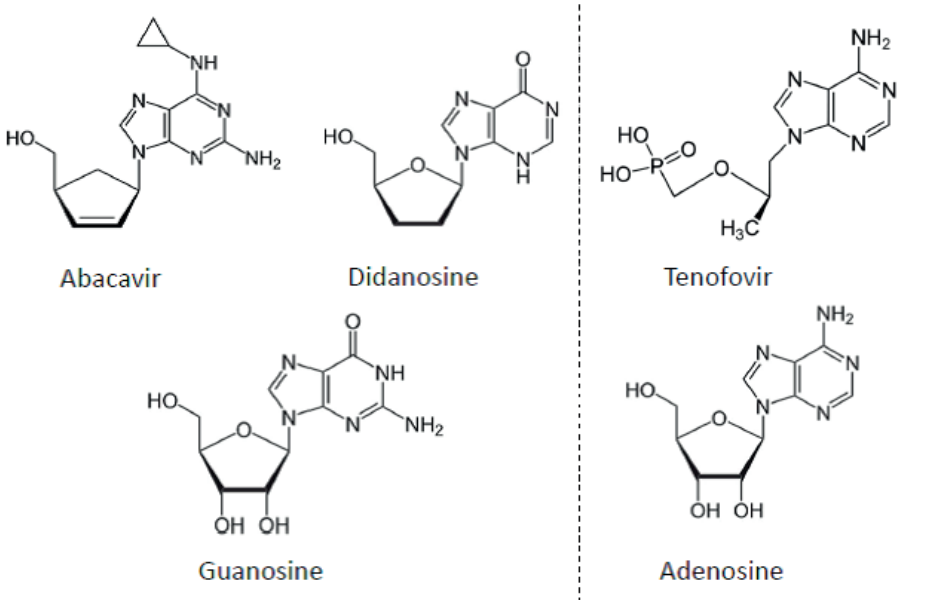

Figure 7. Chemical structures of the purine analogues non-reverse transcriptase inhibitors, abacavir, didanosine and tenofovir and their resemblance with the natural purine nucleosides guanosine and adenosine.

Non-canonical nucleotides

Besides the canonical purine nucleobases (adenine and guanine), other non-canonical nucleobases exist that are not building blocks of the genetic code. For instance, xanthine and hypoxanthine are important non-coding intermediates in purine metabolism. Their respective ribonucleosides are xanthosine and inosine (see Figures 6 and 8). There are also extraordinary metabolites like 8-oxo-deoxyguanosine that are damaged, potentially harmful, non-canonical purines.

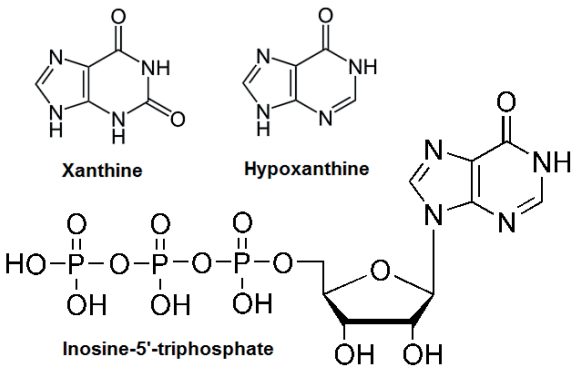

Figure 8. Chemical structures of the non-canonical purine nucleobases Xanthine and Hypoxanthine and the nucleotide Inosine 5'-triphosphate.

The non-canonical nucleotides are formed when canonical nucleotides are damaged by oxidative stress and deamination.[39-41] While the role of the canonical adenine and guanine based nucleotides is extensively studied, the potential role of the non-canonical purine based nucleotides xanthosine and inosine in the human metabolism has not yet been fully clarified and may be both beneficial and harmful. Inosine, inosine 5'-triphosphate (ITP) and to a lesser extend also inosine 5'-diphosphate (IDP) and IMP were found to have anti-inflammatory effects.[42-44] Cyclic ITP (cITP) may function as a second messenger.[45] On the other hand, incorporation of deaminated nucleotides like deoxy-Inosine 5`-triphospate (dITP) and deoxy-Xanthosine 5`-triphosphate (dXTP) into DNA and RNA is thought to have mutagenic consequences.[46,47] Multiple enzymes have been described that clear the cellular nucleotide pool from these potential harmful non-canonical nucleotides.[48] One of those enzymes is Inosine 5'-triphosphate pyrophosphohydrolase (Inosine triphosphatase or ITPase), which dephosphorylates ITP to IMP and xanthosine 5`-triphosphate (XTP) to xanthosine 5`-monophosphate (XMP). IMP and XMP are further processed to the canonical nucleotides AMP and GMP as described above (Figures 5 and 6).

INOSINE TRIPHOSPHATE PYROPHOSPHOHYDROLASE (ITPASE)

Substrates, expression and function

In 1964 Liakopoulou and Alivisatos were the first to describe this enzyme in human erythrocytes, found it to be very specific for ITP[49] and called it ITP phosphohydrolase (ITPase). Their initial conclusion that ITPase hydrolysed ITP to IDP and further to IMP was refuted by Vanderheiden, who showed in his experiments that IDP was not involved in the reaction and that ITPase *pyro*phosphohydrolysed ITP to IMP.[50] The natural substrates for ITPase are ITP, dITP and XTP.[51] Potentially the enzyme is somewhat promiscuous, for (d)GTP, (d)ATP, (d)CTP, TTP and UTP were found in some assays to be pyrophosphohydrolysed by ITPase, although 10-100 fold less efficient.[51] It was shown that (d)IMP and (d)IDP are no substrates at all, and that IDP is in fact an inhibitor of ITPase.[52] The specificity of ITPase for (d)ITP and XTP is unusually high compared to other enzymes of the nucleotide metabolism[53] and this is probably due to the structure of the enzyme (Figure 9).[54] ITPase consists of two monomers of 21.5 kDa, composed of 194 amino acids, forming a dimer. Each monomer consists of a long central β-sheet forming the floor of the active site, with an upper and a lower lobe, between which the substrate binds. The substrate specificity for ITP and XTP is explained by the hydrogen bonds they can form with the active site, while ATP and GTP have amino-groups in those binding locations, which do not allow hydrogen bonding.[55] Because neither of the ribose hydroxyl groups make strong contacts with the protein, ITPase can utilize both ITP as well as dITP.[55]

ITPase is not only expressed in erythrocytes but in a wide range of human tissues (from leukocytes, bone marrow and lymph nodes to solid organs, skeletal muscle, spinal cord and reproductive organs).[51,52] Both the specificity of the enzyme for (d)ITP and XTP and the fact that ITPase is more or less ubiquitously expressed in human tissues are consistent with the hypothesis that ITPase cleans the nucleotide pool from potential harmful nucleotides. Indeed, in embryos of mice with no ITPase activity, eight times higher levels of deoxy Inosine were found in the nuclear DNA compared to control mice embryos. Furthermore, the embryonic fibroblasts of these ITPase devoid mice embryos showed increased chromosome aberrations and accumulation of single-strand breaks in the nuclear DNA.[46]

Genetic basis of ITPase deficiency

In 1969 Vanderheiden measured elevated levels of ITP in the erythrocytes of different families. He suggested that these high levels of ITP were due to ITPase deficiency and that this deficiency was an inheritable trait.[56] In 1980, analysis of a de novo translocation between chromosomes X and 20 of a 13-year-old female showed that the gene *ITPA* encoding for ITPase was appointed to the short arm of chromosome 20.[57] However, it was not until 2001 that the *ITPA* gene was cloned and characterized.[51] One year later five single nucleotide

polymorphisms (SNPs) in this gene were identified. Three silent SNPs (138G>A, 561G>A and 708G>A) and two SNPs that lead to decreased ITPase activity: 94C>A (p.Pro32Thr, rs1127354) and 124+21A>C (or IVS2+21A>C, rs7270101). The 94C>A missense mutation leads to a substitution of proline to threonine amino acid at codon 32. This substitution disrupts the protein structure (Figure 9) and also leads to missplicing of exons 2 and 3.[58,59] Only homozygotes for the 94C>A SNP have nearly complete ITPase deficiency in erythrocytes, in the 94C>A heterozygotes 23% of the ITPase activity remains.[58] The 124+21A>C SNP leads to a less severe state of ITPase deficiency, by miss-splicing exon 3 only,[59] resulting in 60% of mean ITPase activity in heterozygotes and 30% in homozygotes.[58,60] The distribution of the *ITPA* SNPs varies within the different world populations. The 94C>A mutation is highest in Asian populations (14-19%) compared to Caucasian/African populations (6-7%) and Central/South American (1-2%).[61] The 124+21A>C SNP is extremely rare in Asian populations,[62,63] but more frequent in Caucasian populations (11-13%).[58,64,65] Since then, more SNPs have been identified, with varying degrees in residual ITPase activity.[60,62,66]

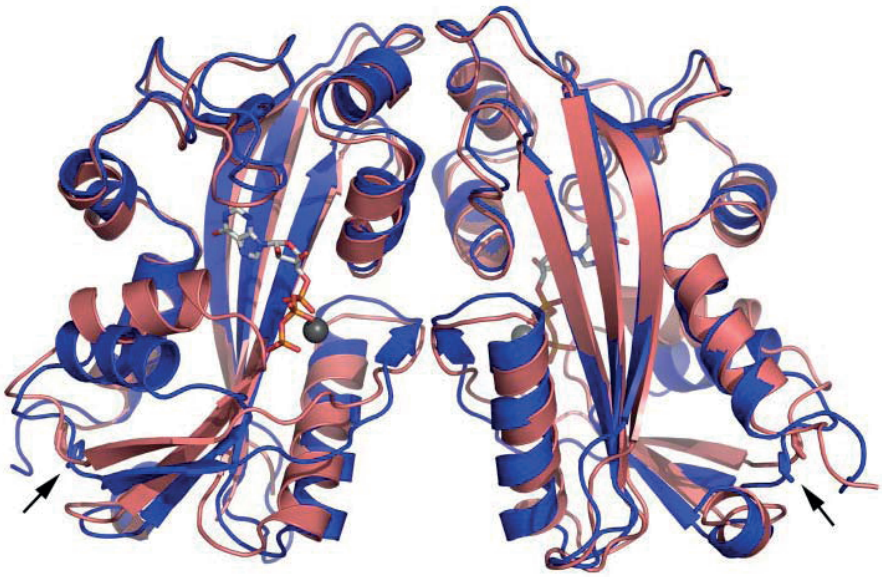

Figure 9. Structure of the Inosine triphosphate pyrophosphohydrolase enzyme. In blue: the unbound (apo) structure, in pink: the ITP-bound structure. The site of the SNP 94C>A (p.Pro32Thr, rs1127354) which leads to a decreased activity of the enzyme is indicated by an arrow for both monomers. *Adapted from: Stenmark, P. et al. Crystal structure of human inosine triphophatase. Substrate binding and implication of the inosine triphosphatase deficiency mutation P32T. The Journal of Biological Chemistry 2006, 282(5): 3182-3187.*

Phenotypic correlation of ITPase deficiency with human diseases

Over the years, multiple studies have been done to find phenotypic abnormalities in individuals with SNPs in the *ITPA* genotype or a decreased ITPase activity. The first to report an increased incidence (16%) of 'High ITP' in a mentally retarded population versus a population with normal intelligence (3%), was Fraser[67] in 1975. Additionally, in chronic paranoid schizophrenic patients a significant decrease in ITPase activity was found compared to a non-psychiatric control population.[68] In 2015 the association between Early Infantile Encephalopathy was found in 7 patients with several pathogenic mutations in *ITPA* that led to a severe degree of ITPase deficiency not restricted to erythrocytes.[66] And recently, in 2018, two families with a very distinctive clinical presentation of lethal infantile-onset dilated cardiomyopathy and the rare Martsolf syndrome, which is characterized by congenital cataracts, postnatal microcephaly, developmental delay, hypotonia and short stature, were found to have mutations in the *ITPA* gene leading to undetectable ITPase protein.[69]

A connection between *ITPA* mutations and malignancy has been hypothesized, although studies investigating this subject are still sparse. In one study an increase in mitochondrial DNA mutations was found in adult hematology patients (consisting of myelodysplastic syndrome, acute myeloid leukemia and chronic lymphocytic leukemia patients), carrying the 94C>A SNP, compared to patients carrying wild-type *ITPA*.[70] In another study *ITPA* is mentioned as one of the genes mutated in adenocarcinoma of the pancreas.[71]

One study reported on an unusually high prevalence of decreased ITPase activity and *ITPA* genotypes with SNPs in patients with pulmonary Langerhans' cell histiocytosis compared to a reference population (50% versus 11% respectively).[72]

THE ROLE OF *ITPA* SNPs IN DRUG METABOLISM

ITPA SNPs have been found to influence drug metabolism of azathioprine and its active metabolite mercaptopurine, methotrexate and ribavirin. Azathioprine is used as an immunosuppressive drug prescribed after organ transplantation, and for multiple autoimmune diseases, like inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus. Azathioprine is metabolized to mercaptopurine (6-MP) which is then further metabolized by multiple enzymatic steps of the purine salvage and interconversion pathways, including ITPase. In 2004, an association between adverse drug reactions of azathioprine such as influenza-like symptoms, rash and pancreatitis was described for the *ITPA* SNP 94C>A in a cohort of patients treated for inflammatory bowel disease.[73] However, these findings could not be confirmed in another study.[74] Since then, some studies reported an association between the 94C>A SNP and bone marrow depression such as decreased

leukocytes[75-77] or agranulocytosis,[78] flu-like symptoms[79] or arthralgia,[80] but in other studies no association could be demonstrated[81-84] in patients with inflammatory bowel disease. Also the outcome of azathioprine therapy was not clearly associated with *ITPA* SNPs.[80,85] SNP 124+21A>C was in all the studies but one[86] not associated with adverse events.[75,77,80,81,83,84] In patients with systemic lupus erythematosus (SLE) 94C>A was associated with a better response to low-dose azathioprine therapy,[87,88] but in patients with renal or liver transplants studies were inconclusive.[89-91] Taken together, in adult patients treated with azathioprine, the SNP 94C>A is associated with adverse events, although not undisputed.

In children treated with mercaptopurine (6-MP) for acute lymphoblastic leukemia the SNP 94C>A in the *ITPA* gene was associated with hepatic toxicity,[92-94] decreased leukocytes[94-96] and decreased event-free survival,[97] although again, these findings were not undisputed, as another study found the opposite effect on event-free survival in a comparable group of patients.[98] In patients treated with methotrexate for rheumatoid arthritis the SNP 94C>A a possible association with a worse clinical response was found.[99,100]

ITPA, ITPASE AND INFECTIOUS DISEASES

Hepatitis C

In patients treated with PEG-Interferon gamma and ribavirin for a hepatitis C virus (HCV) infection, the effect of SNPs in the *ITPA* gene on the occurrence of anemia and hemoglobin (Hb) decline is extensively studied.[101-107] In a meta-analysis by Pineda-Tenor in 2015,[108] the SNPs 94C>A, 124+21A>C and rs6051702 were all associated with protection against Hb decline. Furthermore, 94C>A was also significantly associated with protection against the occurrence of severe anemia and the necessity for ribavirin dose reduction during therapy. None of these studies investigated the association between ITPase activity and the occurrence of anemia and the degree of Hb decline.

HIV

In spite of a genotype distribution comparable to a non-HIV infected population, ITPase activity was found to be significantly decreased in erythrocytes of HIV-infected patients compared to a non-HIV infected control population.[64] However, no further studies were done to investigate whether this affected the occurrence of adverse events during the use of cART. For replication, HIV depends on the nucleotide metabolism of the infected host. For instance, HIV uses reverse transcriptase to convert its single-stranded RNA into double-stranded DNA, using the nucleotides available in the human cell, and further transcribes new RNA from this DNA after incorporation into the human genome, using the human nucleotide mechanism. The NRTIs are analogues of the natural ribonucleotides and

compete with these natural ribonucleotides for incorporation in the growing RNA chain. When incorporated, further RNA synthesis is stopped (chain termination) because of the NRTIs miss a 3'-hydroxyl group on the deoxyribose. To further improve HIV therapy by decreasing adverse events, more insight into the association between *ITPA* genotypes and ITPase activity on the one hand and adverse events during use of cART on the other hand could be important. Anti-retroviral drugs mimicking all nucleobases exist, but since ITPase is an enzyme in the human purine metabolism, we concentrated on the drugs abacavir (a guanosine analogue) and didanosine and tenofovir (adenosine analogues).

OUTLINE OF THE THESIS

The general aim of this thesis is to investigate the influence of the *ITPA* genetic SNPs 94C>A and 124+21A>C and the activity of the enzyme ITPase on the occurrence of adverse events during treatment for the infectious diseases HCV and HIV. While *ITPA* SNPs were previously found to be protective against hemolytic anemia during therapy with ribavirin for HCV, it is not clear to what extent other SNPs contribute to ITPase activity. Therefore, in **Chapter 2**, ITPase activity is compared with *ITPA* genotype for prediction of anemia during ribavirin use for HCV.

In HIV-infected patients the ITPase activity in erythrocytes was found to be decreased compared to a population not infected with HIV. As leukocytes are the main target cells for HIV-infection, in **Chapter 3** the expression of the ITPase protein in leukocytes and leukocyte subpopulations is explored in association with *ITPA* genotype. The results in HIV-infected patients are compared to a non-HIV infected population.

As the current therapy for HIV is highly effective, the main obstacle for treatment nowadays is adverse events during cART. In **Chapter 4**, we investigate the association of ITPase activity and *ITPA* genotype with the occurrence of adverse events during combination antiretroviral therapy for HIV. One of the most important adverse drug events during tenofovir use is nephrotoxicity. In order to test if ITPase activity and *ITPA* genotype could be used as a biomarker to predict nephrotoxicity during tenofovir use for HIV,these parameters are determined and compared in HIV-infected patients with and without nephrotoxicity, in **Chapter 5**.

Other important aspects brought on by the current effective HIV therapy, are long term consequences of cART and increasing diseases of older age, one of them being cardiovascular diseases. The association of ITPase activity and *ITPA* genotype with the occurrence

of cardiovascular diseases and metabolic events during cART for HIV-infection is further explored in **Chapter 6.**

To further unravel effects caused by HIV-infection itself and effects caused by cART, in **Chapter 7**, basic cell metabolomics of untreated HIV-infected patients are compared to a control population (the effect of HIV-infection). In addition metabolomics data prior to start of cART were compared to 12 months of therapy successfully suppressing HIV replication (the effect of cART).

In **Chapter 8** the results of these studies are discussed with respect to future perspectives.

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