The role of PTEN in innate and adaptive immunity

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ABSTRACT

The lipid and protein phosphatase PTEN controls the differentiation and activation of multiple immune cells. PTEN acts downstream of T and B cell receptors, costimulatory molecules, cytokine receptors, integrins, and also growth factor receptors. Loss of PTEN activity in human and mice is associated with cellular and humoral immune dysfunction, lymphoid hyperplasia, and autoimmunity. While most patients with PTEN hamartoma tumour syndrome (PHTS) have no immunological symptoms, a subclinical immune dysfunction is present in many and clinical immunodeficiency in few. Comparison of the immune phenotype caused by PTEN haploinsufficiency in PHTS, phosphoinositide 3-kinase (PI3K) gain of function in activated PI3K syndrome, and mice with conditional biallelic *Pten* deletion suggests a threshold model where coordinated activity of several phosphatases control the PI3K signalling in a cell-type specific manner. Emerging evidence highlights the role of PTEN in polygenic autoimmune disorders, infection, and the immunological response to cancer. Targeting the PI3K axis is an emerging therapeutic avenue.

Keywords: Bannayan Riley Ruvalcaba syndrome, Cowden syndrome, *PTEN* hamartoma tumour syndrome

Abbreviations: PHTS – *PTEN* hamartoma tumour syndrome; PTEN – phosphatase and tensin homolog deleted on chromosome 10, mTOR –mammalian target of rapamycin,

INTRODUCTION

Phosphatase and tensin homolog (*PTEN*) was identified in 1997 as a tumour suppressor gene at the 10q23 locus (Li et al. 1997; Steck et al. 1997). It emerged as a key negative regulator of cellular growth and proliferation and is one of most frequently mutated genes in cancer. Germline *PTEN* mutations can cause a spectrum of autosomal dominant syndromes including Cowden's disease and Bannayan-Riley-Ruvalcaba syndrome that are summarised as PTEN hamartoma tumour syndrome (PHTS) (Yehia et al. 2019).

Whereas most studies have focused on the role of PTEN as a tumour suppressor, PTEN has also an important role in maintaining immune homeostasis as suggested by autoimmunity, lymphoproliferation and immunodeficiency in the absence of PTEN (Chen et al. 2017; Eissing et al. 2018; Fruman et al. 2017). We discuss the role of phosphoinositide 3-kinase (PI3K) and PTEN in signalling downstream of multiple immune receptors and the function of PTEN in different immune cells. We review immunological manifestations in patients with germline PTEN mutations or aberrant PI3K signalling and consider the contribution of PTEN to polygenic immunopathology. Finally, we will assess treatment options that emerge for patients with activation in the PI3K signalling pathway.

PTEN AND IMMUNE RECEPTOR SIGNALLING

PTEN regulates PI3K activation downstream of immune receptors

PTEN encodes a ubiquitously expressed dual specificity phosphatase which acts as a key regulator of cell signalling (Lee et al. 2018). Three different mechanisms for PTEN activity have been described: lipid phosphatase activity, protein phosphatase activity, and additional phosphatase independent functions (Figure 1). PTENs canonical role is negative regulation of the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway through its lipid phosphatase activity, where PTEN directly antagonises the action of PI3K.

In addition to the classical isoform that has been studied extensively, alternative initiation of *PTEN* translation can produce an isoform with a 173 amino-acid N-terminal extension (Liang et al. 2014). This longer variant (PTEN-L) contains a secretion signal and retains lipid and protein phosphatase function when taken up by local cells allowing paracrine signalling (Hopkins et al. 2013; Cao et al. 2018).

PI3Ks are a family of lipid kinases that all serve to phosphorylate the 3' position of phosphatidyl inositol (PI). The Class I group convert PI (4,5) diphosphate into PI (3,4,5) triphosphate (PIP_3) regulating cell growth, survival and glucose metabolism. The Class II and III group generate PI (4,5) P_2 and PI (3) P, the latter key for endosomal trafficking (Okkenhaug 2013).

There are four class I PI3Ks, which are distinguished by their p110 catalytic subunit. In immune cells the p110δ and p110γ isoforms predominate (Okkenhaug 2013; Lucas et al. 2016; Fruman et al. 2017). P110δ associates with the p85α regulatory subunit and is de-repressed by binding to the phosphorylated tyrosine residue of an activated non-receptor tyrosine kinase (Vanhaesebroeck et al. 2010; Okkenhaug 2013). These include the T cell receptor (TCR) and B cell receptor (BCR) signalling complexes. Similarly, co-receptors on T and B cells (i.e. CD28 or ICOS and CD19 or BCAP respectively), multiple Fc receptors (FcγRIIA, FcεRI), co-stimulatory receptors (CD40), cytokine receptors (IL-2R, IL-4R, IL-7R, IL-15R, GM-CSF receptor), integrin receptors, and Toll like receptors (TLR4) also activate PI3Kδ signalling (Hawkins and Stephens 2015; Lucas et al. 2016; Mócsai et al. 2010).

The p110γ isoform is most highly expressed in myeloid cells and couples to the regulatory subunits p87 (also known as p84) or p101 (Hawkins and Stephens 2015). p87 is preferentially activated by direct interaction with G protein coupled receptors , while p101 is activated by Ras binding downstream of G protein coupled receptors, receptor tyrosine kinases, non-receptor tyrosine kinases, and Toll-interleukin 1 receptor (TIR) domain containing receptors (Schmid et al. 2011; Vanhaesebroeck et al. 2010). These regulatory subunits allow initiation of PI3Kγ signalling by a large number of G protein coupled receptor agonists including chemokines (through CXCR and CCR receptors), formyl-peptides, activation of complement receptors via C5a and C3a, and bioactive lipids including sphingosine 1 phosphate, leukotrienes, and prostaglandins (Sun and Ye 2012).

Immune signalling downstream of PI3K

PI3K activation results in recruitment and activation of multiple signalling intermediates that bind to PIP_3 with a pleckstrin homology (PH) domain. The TEC family of tyrosine kinases (ITK, RLK, TEC, and BTK) are PI3K targets that are specific to the adaptive immune system, where they regulate Phospholipase Cγ1 dependent calcium signalling downstream of antigen receptors. Murine and human B cell differentiation is blocked by loss

of function variants of the BTK, that cause X-linked agammaglobulinemia in humans (Fruman et al. 2017). In T cells ITK, RLK and TEC have redundant roles, mice that lack the former two have preserved T cells but with developmental and functional defects (Schwartzberg et al. 2005).

The best-characterised downstream effector of PI3K signalling is the serine/threonine kinase protein kinase B also known as AKT. AKT is recruited to the plasma membrane by PIP_3. AKT has over 100 known substrates that collectively regulate cell growth, survival and glucose metabolism; three of the most important downstream signalling nodes are the mammalian target of rapamycin complex 1 (mTORC1), Glycogen synthase 3 (GSK3), and the Forkhead Box O (FOXO) transcription factors (Manning and Toker 2017) (Figure 1).

mTORC1 is activated downstream of AKT and Tuberous Sclerosis Complex 2 (TSC2). Its substrates include ribosomal S6 kinase (p70-S6K) and eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) which promote protein synthesis; and the transcription factors hypoxia inducible factor 1a (HIF1a) and c-Myc which promote glycolytic cellular metabolism (Song et al. 2012; Okkenhaug 2013; Verbist et al. 2016). This metabolic checkpoint has an important role in determining immune cell differentiation and function (Dang et al. 2011; Newton et al. 2016; Weichhart et al. 2015). The mTORC1 complex also influences immune cells through important negative feedback loops to upstream PI3K and MAPK pathways (Carracedo et al. 2008; Fruman et al. 2017).

GSK3 is inhibited by AKT activity (Manning and Toker 2017). In innate immune cells it promotes inflammatory cytokine production downstream of TLRs, and it has a role in T cell differentiation and function (Beurel et al. 2010). GSK3 also initiates a feedback loop involving miR17-92 and PTEN, which has an important role in B cell development (Benhamou et al. 2016, 2018).

The nuclear localisation of the Foxo transcription factors are regulated by AKT (Brunet et al. 1999). In lymphocytes they regulate expression of important genes for immune cell development, differentiation, and homing such as *Rag1*, *Rag2*, *IL7R*, *EDG1*, *EDG6*, and *FoxP3* (Okkenhaug 2013; Fabre et al. 2008; Newton and Turka 2012).

Protein phosphatase and phosphatase independent activity of PTEN

In addition to regulating the PI3K/AKT/mTOR pathway, PTEN has several lipid phosphatase independent functions. It has protein phosphatase activity against several targets including focal adhesion kinase, which regulates cell migration and is important in anti-tumour immunity (Alfieri et al. 2017); and interferon regulatory factor-3 (IRF3), a

transcription factor for interferon-β which promotes anti-viral immunity and regulates inflammation (Li et al. 2016).

PTEN is also known to have a phosphatase independent role maintaining genomic stability in the cell nucleus, and acting as a scaffold protein in the nucleus and cytoplasm (Lee et al. 2018).

PTEN IN IMMUNE CELLS

The differential expression of immune receptors and the downstream signalling machinery by different immune cells explains the cell type specific functions of regulatory elements such as PTEN. Heterozygous loss of *Pten* in mice and humans results in lymphoproliferative disease, autoimmunity and dysgammaglobulinemia (Di Cristofano 1999; Chen et al. 2017). Germline deletion of both *Pten* alleles is not compatible with life but conditional mouse models allow to explore the role biallelic loss of PTEN in different immune cells (Table 1). Those models provide insight how cells with complete PTEN deficiency respond in an organism with otherwise normal PTEN activity.

Lymphocytes

T cells activation and differentiation is driven by T cell receptor signalling, costimulatory molecules as well as cytokines that activate the Jak/STAT pathway but also PI3K signalling. T cell restricted *Pten* deletion results in mice with severe autoimmune disease, fatal lymphoproliferative disease, and hypergammaglobulinemia (Suzuki et al. 2001; Buckler et al. 2006). Those T cells have increased baseline PI3K pathway activity and hyperactive TCR signalling which is sufficient to overcome the need for co-stimulation and causes defective central and peripheral tolerance, resistance to apoptosis, and increased proliferative signalling (Suzuki et al. 2001; Buckler et al. 2006). By contrast, *Pten* deletion targeted to mature activated T cells results in healthy mice without autoimmune disease or lymphoma, but with enhanced anti-bacterial and anti-tumour immunity (Soond et al. 2012a).

Regulatory T cell (T_{reg}) characterised by the expression of the transcription factor Foxp3 depend on interleukin (IL) -2 a potent activator of PI3K. Mice with T_{reg} restricted loss of PTEN develop severe multi-organ autoimmune disease and lymphoproliferative disease (Huynh et al. 2015; Shrestha et al. 2015). *Pten* deficient T_{reg} cells transition towards an effector phenotype (Huynh et al. 2015). This causes an expansion of $T_{follicular\text{-}helper}$ cell driving B cell class switch recombination, B cell activation, and altered immunoglobulin profiles

(Shrestha et al. 2015). Foxo regulates Foxp3 explaining a close relationship between these two factors (Chen et al. 2017). As the Foxo transcription factors are regulated by AKT, interfering with this axis by introducing constitutively active AKT or deleting Foxo1 results in impaired Treg differentiation, severe autoimmunity, and lymphoproliferative disease (Crellin et al. 2007; Haxhinasto et al. 2008; Ouyang et al. 2012).

T helper type 17 (Th17) cells that are critical for defence against bacteria and fungi. PTEN deficiency restricted to the Th17 lineage impairs Th17 differentiation associated with increased production of IL-2, a known inhibitor of Th17 differentiation (Kim et al. 2017). Those mice are resistant to models of autoimmune disease.

PTEN loss in B cells results in production of autoantibodies, expansion of the B1 and marginal zone populations, and impaired class-switch recombination but not lymphoproliferative disease (Anzelon et al. 2003; Suzuki et al. 2003). As in T cells, loss of PTEN resulted in hyper-responsive antigen receptor signalling which compensated for lack of co-receptor signalling, resulting in inappropriate B cell activation and impaired tolerance (Anzelon et al. 2003). PTEN regulates Foxo-mediated transcription of activation-induced cytidine deaminase (AID), which is required for class switch recombination (Dengler et al. 2008; Suzuki et al. 2003; Omori et al. 2006; Wang et al. 2018b). Regulation of Foxo and expression CXCR4 contributes to defective B cell development via aberrant trafficking in the germinal centre (Dominguez-Sola et al. 2015; Sander et al. 2015).

In Natural Killer (NK) cells, the PI3K pathway promotes target cell or IL15 induced proliferation, pro-inflammatory cytokine production, and perforin and granzyme mediated cytotoxicity (Tassi et al. 2007; Nandagopal et al. 2014; Guo et al. 2008). Concordantly, overexpression of PTEN inhibits cytolytic function and loss of PTEN enhances it (Briercheck et al. 2015; Mace 2018). Mice with NK restricted *Pten* deletion have NK cells with increased proliferative capacity but impaired trafficking (Leong et al. 2015).

It needs to be shown whether PTEN has additional functions in the emerging family of innate lymphoid cells.

Myeloid Cells

In contrast to lymphocytes, where unrestrained PI3K activation causes an overactive immune phenotype, PI3K signalling often drives an anti-inflammatory response in myeloid cells (Vergadi et al. 2017). Models with myeloid specific *Pten* deletions (Table 1) highlight the context specific role of Pten in myeloid cells.

Neutrophil chemotaxis, migration, and effector functions require PI3K activation (Damoulakis et al. 2014; Sasaki et al. 2000; Deladeriere et al. 2015; Condliffe et al. 2005). Myeloid *Pten* deletion enhanced neutrophil chemotaxis, superoxide production, and phagocytosis in some models (Li et al. 2009; Subramanian et al. 2007). In neutropenia-associated pneumonia this reduced mortality (Li et al. 2009). However, this was not replicated in neutrophil-specific Pten deletion (Heit et al. 2008), and further investigation suggests neutrophil recruitment is impaired in vivo (Schabbauer et al. 2010; Heit et al. 2008).

Macrophages require PI3K signalling for chemotaxis and phagocytosis (Zhang et al. 2009; Nishio et al. 2007; Shiratsuchi and Basson 2007). Enhanced chemotaxis and phagocytosis by myeloid loss associated with reduced pro-inflammatory and increased anti-inflammatory cytokines expressed by *Pten* deficient macrophages resulted in increased survival in a murine model of pneumonia (Schabbauer et al. 2010). Anti-inflammatory PI3K signalling has also been noted in models of liver reperfusion injury (Zhu et al. 2017) and many in-vitro studies of the TLR4 PI3K axis (Luyendyk et al. 2008; Androulidaki et al. 2009; Cao et al. 2004; Martin et al. 2005; Chaurasia et al. 2010; Kaneda et al. 2016; Günzl et al. 2010; Sahin et al. 2014) although this might be context specific (Kral et al. 2016; Li et al. 2009).

The effect of *Pten* on macrophage cytokine expression is also isoform specific; PTEN-L has a distinct influence on cytokine production compared to endogenous PTEN, suggesting a mechanism for paracrine modulation of the immunological environment (Cao et al. 2018).

Myeloid *Pten* deletion reduces pro-inflammatory cytokine production by dendritic cells (DCs). This suppressed Th17 activation and onset of inflammation in a T cell dependent model of collagen induced arthritis, whereas disease severity in the K/BxN serum transfer arthritis (which does not require an adaptive immune response) was not affected (Blüml et al. 2015). Myeloid PTEN deletion alters DC function, resulting in impaired activation of $CD8^+$ T cells and reduced survival in a colitis associated cancer model (Kuttke et al. 2016). Interestingly, DC specific *Pten* deletion had a wider impact on the innate immune system since it decreased neutrophil frequency and myeloproliferative disease (Jiao et al. 2014).

IMMUNE DYSFUNCTION IN HUMANS WITH REDUCED PTEN ACTIVITY

The multiple immune cell-specific abnormalities that result from dysregulated PI3K signalling were first identified using murine models followed by patient studies. Increased PI3K signalling is a hallmark of patients with PTEN hamartoma tumour syndrome (PHTS), caused by heterozygous germline *PTEN* mutations, and activated PI3K syndrome (APDS), caused by gain of function mutation in *PIK3CD* (encodes p110δ, APDS1) or *PIK3R1* (encodes p85α, APDS2). Investigation of the immunological phenotype of these patients has furthered understanding of the role of PTEN in human immunity.

The immune phenotype of PHTS

PHTS groups several overlapping clinical syndromes including Cowden's syndrome and Bannayan Riley Ruvalcaba syndrome. Diagnostic clinical features of PHTS are macrocephaly, developmental delay or autistic spectrum disorder, history of cancer; as well as various skin, vascular, and mucosal anomalies. Patients are at increased risk of breast, thyroid, renal, and endometrial cancer, or hamartomas and benign lesions (Cleveland Clinic PTEN risk score) (Mester and Eng 2015).

A spectrum of immune dysfunction ranging from asymptomatic lymphopenia in the peripheral blood, lymphoid hyperplasia, autoimmunity, and immunodeficiency have been identified in patients with confirmed *PTEN* mutations (Table 2). Those features have incomplete penetrance and variable expressivity.

The majority of patients with *PHTS* do not have immunological disease, i.e. infection susceptibility or inflammation but many show evidence of subclinical immunological abnormalities such as reduced total numbers of $CD4^+$ T cells, reduced CD4:CD8 ratio or altered immunoglobulin subclasses (Heindl et al. 2012; Chen et al. 2017).

Lymphoid hyperplasia is a common finding in patients with PHTS (Table 2). In a case series of 79 patients 24% had lymphoid hyperplasia (Chen et al. 2017). There is a variable severity ranging from asymptomatic and clinically not relevant intestinal lymphoid hyperplasia toward severe and potentially life threatening adenoid lymphoid hyperplasia or thymus hyperplasia (Sharma et al. 2007; Tsujita et al. 2016; Heindl et al. 2012).

Autoimmune diseases including Hashimoto's thyroiditis, coeliac disease and autoimmune colitis, and autoimmune anaemia was reported in 29% of 79 PHTS patients

(Table 2) (Chen et al. 2017). It has also been suggested that patients with PHTS may have higher prevalence of eosinophilic gastrointestinal disorders (Henderson et al. 2014).

Clinical immunodeficiency is a rare but consistent feature in PHTS, so far only described in eight patients with confirmed *PTEN* mutation (Table 2). However further patients with immunodeficiency had a clinical diagnosis of Cowden syndrome (Kang et al. 2009; Ruschak 1981; Amer et al. 2011). Common variable immunodeficiency (CVID) with recurrent respiratory tract infections and hypogammaglobulinemia was diagnosed in five PHTS patients. This was associated with impaired class switch recombination and somatic hypermutation, and elevated transitional B cell count (Table 2). A severe combined immunodeficiency (SCID) like phenotype was reported in one patient with PHTS; a two-year-old boy who had suffered from recurrent infections including pneumocystis jirovecci pneumonia (Tsujita et al. 2016). He was found to have hepatosplenomegaly, hypergammaglobulinemia with raised IgM, lymphopenia, and a reversed CD4 to CD8 ratio. Notably this patient was found to have markedly lower PTEN expression than other patients with PHTS (11% versus 60%). At least four patients with immunodeficiency have been given immunoglobulin replacement with variable success (Tsujita et al. 2016; Driessen et al. 2016).

It is likely that the immune defects in PHTS are caused by a variety of different but interrelated mechanisms, i.e. dysfunctional lymphocyte activation and metabolism likely causes lymphocyte exhaustion, defective lymphocyte homing contributes to trapping in lymphoid organs and a mild regulatory T cell defect might shift the balance towards autoimmunity.

Comparison between the immune phenotype in PHTS and activated PI3Kδ Syndrome

The immune phenotype of PHTS shows clear similarities to that of activated PI3K syndrome (APDS) which is caused by gain of function variants in *PIK3CD* (encoding p110δ, APDS1) or in *PIK3R1* (encoding p85α, APDS2) (Coulter et al. 2017; Elkaim et al. 2016). Immune dysfunction in APDS is more severe and has higher penetrance (Table 3, Figure 2). APDS patients present with recurrent sinopulmonary infections often leading to bronchiectasis, skin abscesses, and chronic viral infections. Patients typically have reduced $CD4_+$ T cell count, decreased or reversed CD4/CD8 ratio, reduced class switched antibody production, and impaired antibody response to vaccines. Lymphoid hyperplasia is present in over half of APDS cases and can be severe; malignant lymphoproliferative disease developed in 13% of APDS1 and 28% of APDS2 patients (Coulter et al. 2017; Elkaim et al. 2016).

Several patients have been described with autoimmune and autoinflammatory diseases such as autoimmune cytopenia, glomerulonephritis, primary sclerosing cholangitis, and autoimmune hepatitis (Coulter et al. 2017; Hartman et al. 2015; Elkaim et al. 2016).

The comparison between the immune phenotype of PHTS and APDS suggests a direct dose-effect-model where greater PI3K pathway activation in APDS compared to PHTS may account for the more severe immunological phenotype in APDS. In PHTS PTEN expression is around 60% of normal in activated T cells (Chen et al. 2017; Tsujita et al. 2016) resulting in a small increase in PIP_3 and modest increases in the phosphorylation of downstream mediators (Browning et al. 2015; Tsujita et al. 2016). In contrast, augmented PI3Kδ function in APDS can triple PIP_3 production and cause much greater phosphorylation of AKT and downstream mediators (Angulo et al. 2013; Tsujita et al. 2016; Browning et al. 2015). This is consistent with the dose effects observed in animal models and raises the question whether the immunodeficiency in some patients with PHTS is caused by stochastic effects, environmental trigger that shifted the PIP3 balance at a critical time point or due to additional genetic factors that control the PI3K signalling pathway.

PTEN AND POLYGENIC IMMUNE MEDIATED INFLAMMATORY DISEASES

In addition to its obvious role in the PHTS Mendelian group of disorders, there is emerging evidence that PTEN plays a relevant role in polygenetic immune-mediated diseases. Humoral autoimmunity is associated with reduced PTEN expression in B cells and CD4 T cells (Wu et al. 2014; Wang et al. 2018c; Zhao et al. 2019b). In systemic lupus erythematosus (SLE) reduced level of PTEN in hyper-reactive B cells correlates with disease activity, and antagonising the miRNAs responsible for negatively regulating PTEN expression normalised B cell behaviour in vitro (Wu et al. 2014). In immune thrombocytopenia (ITP) PTEN is similarly reduced in B cells, leading to hyperreactivity and increased plasma cell differentiation (Wang et al. 2018c), and is also reduced in autoreactive CD4 Th2 cells (Zhao et al. 2019b). In patients with immune mediated nephritis PTEN-L is downregulated and increasing PTEN-L expression via knock-in mutations resulted in reduced tissue inflammation in a murine model (Wang et al. 2018a). Reduction in PTEN is associated with airway inflammatory disorders such as asthma and COPD, and increased cellular levels of *PTEN* introduced by an adenovirus vector can reduce airway hyper-responsiveness and

inflammation in models of asthma (Yang et al. 2017; Kim et al. 2007; Kwak et al. 2003; Khalifeh-Soltani et al. 2018).

This altogether suggests that the reduced expression levels of PTEN in subsets of immune cells in immune mediated inflammatory disorders is not only a bystander effect. The exact role of PTEN in those polygenic disorders need to be clarified. Although hundreds of loci have been associated by genome-wide linkage studies with autoimmune disorders, PTEN itself has not emerged as a strong candidate locus. It therefore needs to be shown whether the observed transcriptional changes seen in immune mediated disorders are caused by trans-regulating loci or a consequence of indirect regulation via epigenetic regulation.

PTEN CONTROLS THE IMMUNE RESPONSE TO INFECTION

Due to the diverse functions of PTEN in regulating immunity, it is unsurprising that PTEN controls aspects of the inflammatory response to acute bacterial and viral infection. PTEN inhibition in a murine model of sepsis led to increased inflammation, tissue injury, and mortality (Sisti et al. 2018). Increased inflammatory damage was also noted with PTEN-L deletion in a model of *Pseudomonas aeruginosa* infection (Riquelme et al. 2017).

The immune response to viral infection is similarly regulated via PTEN. Viruses induce PTEN upregulation in B cells during acute infection which suppresses the B cell response following BCR and CXCR4 stimulation and impairs the humoral immune response (Getahun et al. 2017). In chronic human immunodeficiency virus (HIV) infection PTEN is similarly upregulated, which impairs memory B cell formation and contributes to deficient anti-viral immunity (de Armas et al. 2019). Conversely, patients with long-term non-progressive viral disease have reduced PTEN expression which promotes $CD8^+$ T cell proliferation, survival and effector functions and likely contributes to their anti-HIV immunity (Yin et al. 2018). Conversely, PTEN promotes innate anti-viral immunity by dephosphorylating IRF-3 (Li et al. 2016), and in a murine model of acute viral infection intravenous administration of PTEN-L lowered viral titres (Cao et al. 2018). Altogether those data suggest that some pathogens might control PTEN expression as a way to increase replication.

IMMUNOLOGICAL ROLE FOR PTEN IN THE TUMOR MICROENVIRONMENT

PTEN mutations in cancer cells play a major role for progression of haematopoietic and non-haematopoietic forms of malignancy (reviewed in chapter xx). This is not only cell intrinsic since it also confers resistance to immunotherapy (Cheng and Eng 2019; Zhao et al.

2019a). PTEN expression in immune cells is also a significant factor in the development and progression of malignancy since it regulates the immune response to cancer. Immune cells within the tumour microenvironment have an reduced PTEN expression. In particular tumour associated macrophages in an immunosuppressive tumour microenvironment show reduced PTEN expression (Li et al. 2015). Indeed cancer cells actively promote this immune phenotype by suppressing PTEN with miRNA (Li et al. 2018). PTEN expression in stromal cells also contributes to an anti-oncogenic microenvironment by suppressing angiogenesis and extra-cellular matrix remodelling (Qin et al. 2018). This raises the question of whether PTEN depletion in cells of the tumour microenvironment in PHTS does contribute to cancer development in these patients. Further investigation is required to fully understand how PTEN influences macrophage polarisation since ex-vivo macrophages from PHTS patients demonstrated pro-inflammatory cytokine production on co-culture with thyroid cancer cell lines (Sloot et al. 2019).

SPATIOTEMPORAL REGULATION OF PTEN AND PHOSPHATASE NETWORK FAILSAFE MECHANISMS

The function of phosphatases such as PTEN is actively regulated in immune cells. This can be achieved through differential gene expression, post-translational modification and recruitment to immune receptors. In addition to its expression, PTEN activity is posttranslational controlled within different subcellular compartments. Furthermore, PTEN acts not in isolation but coordinated activity with other phosphatases. Those phosphatases that intersects at different level of the PI3K pathway include SHP1/2, PHLPP, and PP2. The activity of PTEN and other phosphatases is indeed not only a tonic activity but requires accumulation and activity at the same membrane and non-membrane signalling complexes that are formed by receptor engagement. In T_{reg} cells PHLPP1 mediates AKT dephosphorylation and this can compensate even for PTEN deficiency (Chen et al. 2017; Patterson et al. 2011). Coordinated and localised activity of PTEN and PHLPP at the immunological synapse afforded via the adaptor molecule NHERF prevents hyperactivation of the PI3K signalling pathway downstream of mTORC1 and allows T_{regs} to maintain a normal phenotype in PHTS (Chen et al. 2017). In B cells there is likely additional compensation due to activity of SHP as suggested by animal models (Getahun et al. 2017). Similarly, in B cells ligation of FcyRIIb recruit SHP1 and SHIP1 to its immunoreceptor

tyrosine-based inhibitory motif (ITIM) allowing coordinated focal activity (Getahun and Cambier 2015).

TARGETING THE PI3K PATHWAY IN IMMUNE MEDIATED DISEASES

The PI3K pathway is the genetically informed therapeutic target in PHTS and APDS, and a potential target in polygenic immunological diseases. Whereas targeting PTEN expression via control of the ubiquitin ligase WWP1 is a potential future target (Lee et al. 2019), most treatments control the signalling pathway via the kinase PI3K, upstream or downstream of the PI3K-PTEN pathway were multiple clinically relevant targets have emerged in recent years. Those treatments will be specific at the receptor level and more generic downstream of the PI3K (for an example see Figure 4).

In PHTS, targeting mTOR via rapamycin downstream of PI3K normalised thymic hyperplasia and normalised the frequency of transitional B cells (Chen et al. 2017).

Specific PI3Kδ inhibitors have been used as proof-of-concept in APDS (Michalovich and Nejentsev 2018), and evaluated for use in polygenic immunological diseases such as COPD and rheumatoid arthritis (Cahn et al. 2017; Bartok et al. 2012). PI3Kδ inhibitors are generally well tolerated in cancer treatment, but are known to cause a number of immunological side effects such as autoimmunity, colitis and infection susceptibility to cytomegaly virus (Stark et al. 2015). Whereas this will limit utility in polygenic immunological disorders, off-target immunological effects may be an unavoidable consequence in the cancer setting. Future therapies could achieve a more cell specific effect by targeting downstream mediators or elements of the regulatory network.

CONCLUDING REMARKS

The PI3K signalling pathways condenses a wide range of receptor and metabolic inputs into a narrow core response of reduced complexity, before proliferation and downstream effectors restores a wide range of outputs (Tieri et al. 2010). This architecture allows very efficient response to multiple inputs but leaves the network vulnerable to disruption at its core, which is formed by PI3K and PTEN. Genetic defects in PTEN or PI3K subunits illustrate the importance of PTEN as a central regulator of the PI3K signalling in immune cell. Further research is required to correlate the immune phenotypes of PHTS with the net PTEN activity (gene expression level), its isoform contribution and regulatory phosphatase activity by other phosphatases. Fine-tuning the activity of PTEN regulated

signalling pathways is a promising treatment of immune dysfunction in patients with PHTS and polygenic immunological disease.

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CONFLICT OF INTEREST

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Table1: Phenotypes of Murine Models with Lymphoid and Myeloid PTEN deletion

Genotype & Restriction	Phenotype	Reference
Germline Homozygous *Pten*$^{-/-}$	Incompatible with life	(Suzuki et al. 1998)
Germline Heterozygous *Pten* $^{+/-}$	Lymphoproliferative disease Autoimmunity Hypergammaglobulinemia	(Di Cristofano 1999)
T cells *Lck-Cre Pten* $^{flox/-}$ *CD4-Cre Pten*$^{flox/flox}$	Lymphoproliferative disease Autoimmunity Hypergammaglobulinemia	(Suzuki et al. 2001; Buckler et al. 2006)
Activated T cells *OX40-Cre Pten*$^{flox/flox}$	Increased T cell cytokine production Enhanced cytotoxic and humoral immune responses	(Soond et al. 2012b; Rolf et al. 2010)
Regulatory T cells *Foxp3-Cre Pten*$^{flox/flox}$	Lymphoproliferative disease Autoimmunity Elevated serum IgG2	(Huynh et al. 2015; Shrestha et al. 2015)
Th17 cells *IL17a-Cre Pten*$^{flox/flox}$	Increased IL2 production Reduced Th17 differentiation Reduced inflammation in autoimmune encephalitis model	(Kim et al. 2017)
B cells *CD19-Cre- Pten*$^{flox/flox}$	Expansion of B1 and MZ B cell compartments. Autoantibody production. impaired class switch recombination.	(Suzuki et al. 2003; Anzelon et al. 2003; Omori et al. 2006)
NK cells *Ncr1KI-iCre PTEN*$^{flox/flox}$	Increased NK cell proliferation. Impaired NK cell homing. Impaired NK cell trafficking to intraperitoneal injection of lymphoma cells	(Leong et al. 2015)

Myeloid *Lys M-Cre* $Pten^{flox/flox}$	Neutropenia-associated E coli pneumonia	Enhanced neutrophil recruitment, superoxide production, and phagocytosis. Enhanced macrophage recruitment Increased pro-inflammatory cytokines Reduced disease severity & mortality	(Li et al. 2009)
	Pulmonary sterile LPS	Increased disease severity	
Myeloid *Lys M-Cre* $Pten^{flox/flox}$	Thioglycollate or E coli peritonitis	Enhanced neutrophil recruitment Enhanced neutrophil superoxide production (ex-vivo)	(Subramanian et al. 2007)
Myeloid *Lys M-Cre* $Pten^{flox/flox}$	S pneumoniae pneumonia	Impaired neutrophil recruitment Enhanced macrophage recruitment and phagocytosis Reduced pro-inflammatory cytokines Reduced macrophage CXCR1 production Increased IL-10 Increased bacterial load at 48 hours, but overall reduced lung damage & prolonged survival	(Schabbauer et al. 2010)
	S pneumoniae peritonitis	Enhanced neutrophil recruitment Increased macrophage CXCR1 production	
Myeloid *Lys M-Cre* $Pten^{flox/flox}$	Bleomycin induced pulmonary fibrosis	Increased pro-inflammatory cytokines Increased TGF-B1 Increased disease severity, reduced survival	(Kral et al. 2016)
Myeloid *Lys M-Cre* $Pten^{flox/flox}$	Liver ischemia and reperfusion injury	Reduced macrophage recruitment Reduced pro-inflammatory cytokines Increased TGF-B Increased T_{reg} & reduced Th17 induction Reduced hepatocellular damage	(Zhu et al. 2017)
Myeloid *Lys M-Cre* $Pten^{flox/flox}$	Collagen induced arthritis (CIA)	Reduced pro-inflammatory cytokines Reduced Th17 response Prevented onset of disease	(Blüml et al. 2015)

	K/BxN serum transfer arthritis	No change to disease	
Myeloid *Lys M-Cre* *Pten*$^{flox/flox}$	Colitis associated cancer	CD8a+ DC population is expanded, but cells are functionally immature & have inhibitory phenotype Reduced CD8 T cell response Increased disease severity and reduced survival.	(Kuttke et al. 2016)
Neutrophils Ela2-Cre Pten$^{flox/flox}$	S aureus peritonitis	Defective neutrophil chemotaxis Increased severity and duration of infection	(Heit et al. 2008)
	K/BxN serum-transfer arthritis	Defective neutrophil chemotaxis Reduced severity of inflammation	
Dendritic Cells *CD11c-Cre Pten*$^{flox/flox}$		Myeloproliferative disease Decreased neutrophil frequency	(Jiao et al. 2014)

NK = Natural Killer, MZ = marginal zone, DC = dendritic cell, pro-inflammatory cytokines unless otherwise specified refer to a combination of TNFa, IL-1, IL-6, IL-12

Table 2: Clinical & Immunological features in patients with confirmed PTEN mutations

Study*	n	Dx	**Clinical features**			**Immunological features**	
			Immuno-deficiency	Lymphoid Hyperplasia	Autoimmunity	Cellular	Humoral
Chen et al. 2017; *Heindl et al. 2012*	79	PHTS	not described	24% of cohort moderate	29% of cohort moderate	↓ CD4 count ↓ CD4/CD8 ratio	↑ transitional B cells Hypogammaglobulinemia
Shaco-Levy et al. 2017	12	PHTS	not described	Frequent, mild	not described	-	-
Driessen et al. 2016	9	PHTS	Occasional, moderate (CVID)	not described	not described	↓ CD4/CD8 ratio	↓ class switching ↓ Somatic Hypermutation Hypogammaglobulinemia
Tsujita et al. 2016	4	CS (2/4)	Severe (CVID, other)	Severe	Moderate	↓CD4 count ↓CD4/CD8 ratio	↓ class switching Hypogammaglobulinemia Hypergammaglobulinemia
Browning et al.	2	CS	Moderate	Mild	not described	↓CD4 count	↑ transitional B cells

2015						↓CD4/CD8 ratio	Hypogammaglobulinemia
Sharma et al. 2007	2	BRRS	not described	Severe	not described	-	-
Mauro et al. 2017	1	PHTS	Moderate	Moderate	Moderate	Mild lymphopenia	-
Boccone et al. 2006	1	BRRS	not described	Moderate	not described	not described	not described

**Similar immunological dysfunction has been described in ungenotyped patients with PHTS/CS (Ruschak 1981; Saccardi et al. 1994; Kennedy et al. 2008; Amer et al. 2011; Starink et al. 1986; Guerin et al. 1989; Kang et al. 2009; Halevy et al. 1985).*

Summarised key clinical and immunological information is displayed. Severity is described as severe (life limiting, life changing), moderate (recurrent inpatient treatment or investigation), and mild (clinically apparent, requiring treatment). Frequency is described in case series of >5 patients and unless a percentage is quoted is categorised as frequent (>50%) or occasional (<50%).

Table 3: Clinical & Immunological features in patients with confirmed PI3K mutations

			Clinical features			Immunological features	
Study	**n**	**Dx**	Immuno-deficiency	Lymphoid Hyperplasia	Autoimmunity	Cellular	Humoral
Coulter et al. 2017; *Kracker et al. 2014; Angulo et al. 2013*	53	APDS1	Severe	75% of cohort severe	34% of cohort, severe	Reversed CD4/CD8 ↓ CD4 cells	↑ transitional B cells ↑IgM ↓ class switching
Wentink et al. 2017	13	APDS1 (11) APDS2 (2)	Severe	Occasional, Severe	Occasional, Severe	↓CD4	↑IgM
Lucas et al. 2014	9	APDS1	Severe	Frequent, severe	Occasional, moderate	↓ CD4/CD8	↑ transitional B cells ↓ class switching Hypogammaglobulinemia Hypergammaglobulinemia
Tsujita et al. 2016	5	APDS1	Moderate	Moderate	not described	Normal	↑ transitional B cells
Hartman et al. 2015	5	APDS1	Severe	Moderate	Severe	↓CD4	↑IgM
Crank et al.	3	APDS1	Severe	Severe	Occasional,	↓ CD4/CD8	↑ transitional B cells

2014					moderate		
Jou et al. 2006	1	APDS1	Severe	not described	not described	↓ CD4/CD8	hypogammaglobulinemia
Elkaim et al. 2016 *Deau et al. 2015; Lucas et al. 2014b*	36	APDS2	Severe	89% Severe	17% Severe	Reversed CD4/CD8 ↓ CD4 cells	↑ transitional B cells ↑IgM ↓ class switching

Summarised key clinical and immunological information is displayed. Severity is described as severe (life limiting, life changing), moderate (recurrent inpatient treatment or investigation), and mild (clinically apparent, requiring treatment). Frequency is described in case series of >5 patients and unless a percentage is quoted is categorised as frequent (>50%) or occasional (<50%). Frequency of immunodeficiency is not quoted as patients were identified through screening primary immunodeficiency registers.

Figure legends

Figure 1: PTEN in immune cell signalling

Immune receptors of non-receptor tyrosine kinase and G protein coupled receptors type can activate PI3K through interaction with the p85a and p87 subunits, or indirectly by activating Ras which binds to p101. PTEN directly antagonises PI3K by converting PIP_3 to $PI(4,5)P_2$. Downstream of PIP_3 multiple signalling intermediates act in the cytoplasm and cell nucleus to promote different aspects of leukocyte function and differentiation. PTEN lipid phosphatase independent actions are shown in purple.

Figure 2: Immune phenotypes in PI3K Dysregulation

A comparison of the immunological phenotypes of PHTS and APDS.

Figure 3: Consequences of PTEN defects in different immune cells.

An illustration of various mechanisms by which *Pten* deletion in lymphoid and myeloid cells leads to the immunological phenotype.

Figure 4: Therapeutic manipulation of immune cell signalling via the PI3K pathway.

As an example the IL2 signalling pathway is shown. In addition to its canonical STAT5 signalling, IL2 signalling also mediated PI3K signalling. Whereas the direct manipulation of PTEN expression is an emerging therapeutic via blockade of WWP1, the dysregulated signalling pathway can be targeted at the level of PI3Kd, as well as upstream at the cytokine and cytokine receptor level or downstream at the level of mTOR. Clinical inhibitors of this pathway are indicated. Clinical experience in patients with PHTS or APDS exists with blockade of PI3Kd and mTOR.

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