Potential immunological effects of gender-affirming hormone therapy in transgender people – an unexplored area of research

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Abstract: There are well-described sex-based differences in how the immune system operates. In particular, cisgender (cis) females have a more easily activated immune system; associated with an increased prevalence of autoimmune diseases and adverse events following vaccinations. Conversely, cis males have a higher threshold for immune activation, and are more prone to certain infectious diseases, such as coronavirus disease (COVID-19). Oestrogen and testosterone have immune-modulatory properties, and it is likely that these contribute to the sexual dimorphism of the immune system. There are also important immune-related genes located on the X chromosome, such as toll-like receptor (TLR) 7/8; and the mosaic bi-allelic expression of such genes may contribute to the state of immune hyperactivation in cis females. The scientific literature strongly suggests that sex-based differences in the functioning of the immune system are related to both X-linked genes and immune modulation by sex hormones. However, it is currently not clear how this impacts transgender (trans) people receiving gender-affirming hormonal therapy. Moreover, it is estimated that in Australia, at least 2.3% of adolescents identify as trans and/or gender diverse, and referrals to specialist gender-affirming care are increasing each year. Despite the improving social awareness of trans people, they remain chronically underrepresented in the scientific literature. In addition, a small number of case studies describe new onset autoimmune disorders in adult trans females following oestrogen use. However, there is currently minimal long-term research with an immunological focus on trans people. Therefore, to ensure the positive health outcomes of trans people, it is crucial that the role of sex hormones in immune modulation is investigated further.

Keywords: oestrogen, immunology, sex hormones, testosterone, transgender, trans health

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Foreword
For the purposes of this review, ‘sex’ refers to the genetic state of being male (XY) or female (XX). ‘Gender’ refers to the way in which people may identify as men, women, non-binary or other genders regardless of their phenotypic sex identified at birth. Individuals with a biological sex that is congruent with their gender identity are referred to as cisgender (cis), denoted as cis male or cis female. Whereas, individuals with a gender identity that is incongruent with their biological sex are referred to as transgender (trans). It is indicated where reference is made to trans people using the terms trans male or trans female throughout, according to the person’s self-identified gender: for example, a person, who is genetically XX, who identifies as male, is referred to as a trans male.

Introduction
The immune system is differently regulated in cis males and cis females. In general, cis females display stronger immune responses to infections and...
This corresponds to reduced mortality from infectious disease, but increased risk of side effects from vaccines in cis females. As a result of a more easily activated immune system, cis females also display higher rates of autoimmune disease (Figure 1). For example, systemic lupus erythematosus (SLE) exhibits one of the strongest sex biases and is approximately eight times more common in cis females compared with cis males. Contrastingly, the cis male immune system has a higher threshold for immune activation, resulting in increased infection-induced comorbidities, elevated prevalence of some cancers but lower risk of autoimmune disease. The difference in immune regulation between cis males and cis females is likely multifactorial, and impacted by sex hormones and chromosomal makeup. Cis females are genetically XX, with two copies of the X chromosome in every nucleated cell. To prevent excessive gene expression, one X chromosome is randomly inactivated so that cis females have the same complement of these genes as XY males. However, chromosome inactivation is variable in cis females, which is referred to as ‘X-inactivation silencing’ and is thought to occur in approximately 15% of X-linked genes. The X chromosome contains genes, such as toll-like receptor (TLR) 7, T-cell co-activator CD40 and T-cell transcription factor FoxP3, which are important for the regulation of immune function. Altered expression levels of these genes through X-inactivation silencing may contribute to differential immune regulation between the sexes, however, this relationship is complex and remains incompletely understood. In addition to this, both oestrogen and testosterone have significant immune-modulatory properties, as reviewed. However, it is currently unclear how interactions between X chromosomes and sex hormones may affect the functioning of the immune system. This has special relevance for trans people on gender-affirming hormonal therapy; it is currently unknown whether they have altered immune function, susceptibility to infection or adequate responses following vaccinations. These may have important health implications and necessitates further research.

**Figure 1.** An overview of the difference in disease incidence between cisgender (cis) males and cis females, and the intersection with transgender (trans) people. To date, relatively little is understood regarding the incidence of immune-mediated disease in the trans people.
Transgender people
Gender-affirming hormonal therapy for trans people is diverse. It depends on the specific goals of treatment, and may involve medications to block or reduce the effects of endogenous sex hormones, as well as oestrogen or testosterone to feminise or masculinise the trans person. Despite the increasing appreciation that sex and gender are important biological variables, a systematic review of the literature determined that more than 60% of immunology papers omitted the sex of subjects.20 Similarly, the experiences of trans people are chronically underrepresented in the scientific literature, despite the fact that this may be pertinent to providing gender-affirming hormones to trans people. As such, the long-term health outcomes of trans people receiving gender-affirming care remain poorly researched.21 Important epidemiological data regarding trans people, including response to infection, prevalence of non-communicable diseases and susceptibility to autoimmune diseases, are unknown. It is also worth noting that the existing published trans health research often includes outdated terminology, pathologises trans people, and often incorrectly uses pronouns and gendered language. This is directly contradictory to the increasing social acceptance and awareness of trans people and their experiences, and indicates that there is still a lack of understanding of gender diversity within the scientific community. Historically, research has been done on and not with trans people, and therefore, there is a strong need for trans health research that is respectful, collaborative and directly benefits the lives of trans people.22

The human immune system is sexually dimorphic
The immune system has evolved to recognise a diverse range of pathogens while maintaining tolerance against self.23 It comprises a complex network of cell subsets, cytokine cascades and signalling pathways, which orchestrates fast and effective clearance of pathogens.23 Immune responses involve cross-talk with regulatory processes, so that, pathogens can be neutralised while avoiding side effects to the host.23 In addition, the interplay between the innate and adaptive immune system is crucial for adequate immune function. Both of these branches of the immune system have specialised effector functions to create sustained immunological memory and prevent against reinfections.23

Sex hormones modulate the immune system
Sex hormones control the reproductive system, and in recent years, there is a growing number of publications reporting their regulatory effects on the immune system (Table 1).7 Cells of both the innate and adaptive arm of the immune system express receptors for sex hormones, such as oestrogen and testosterone, eliciting variable responses depending on the stimulus, target cell and hormone concentration.1 Oestrogen and testosterone are produced in both cis males and cis females, however, at substantially different levels. The majority of hormone receptors function as hormone-activated transcription factors that bind to DNA sequences called hormone response elements, to elicit gene expression.18

Concentration-dependent effects of oestrogens
Oestrogens, progesterone, luteinising hormone (LH) and follicle-stimulating hormone (FSH) are the predominant sex hormones in cis females.24 Oestrogen is routinely used as gender-affirming therapy in trans females, and there is an increasing demand for access to progesterone as a combined hormone therapy. However, since there is currently limited research on the potential immune impacts of progesterone, this review will focus on the established effects of oestrogen on immune modulation. Oestrogen receptors (ERs), including ERα and ERβ, are widely expressed in the tissues of both cis males and cis females.25 ER subtypes are variably expressed in immune cells, and signalling via these receptors is important for development of B cells, monocytes, dendritic cells (DCs) and natural killer cells.1 Oestrogens have immune-activating effects, including promoting Th1 cell differentiation that produces pro-inflammatory mediators, such as interferon gamma (IFN-γ), modulating B cell activation26,27 and regulating type I IFN responses in DCs.11,28 These effects have all been linked to disease severity in autoimmune conditions, such as SLE.29–31 During pregnancy, levels of oestrogens increase, and at those concentrations, oestrogens appear to instead have immune inhibitory effects. This may be associated with involution of the thymus that occurs due to the increased levels of oestrogen.1,28,32 The physiological importance
of this association is currently unclear; however, since the maturation of T lymphocytes generally occurs in the thymus, this may be related to the maternal tolerance of a foetus. Increased oestrogen concentration is also associated with promoting Th2 immunity, which may also explain why some autoimmune diseases, such as multiple sclerosis (MS), which is a Th1-mediated disease, improve during pregnancy.33

Androgens are important immune suppressants

Androgens are a class of steroid hormones including testosterone that have ‘masculinising effects’. Testosterone is synthesised in different tissues in cis males and cis females. In cis males, testosterone is converted to the more biologically active form dihydrotestosterone by 5α reductase in the testes, or to oestradiol by aromatase. In cis females, the conversion of testosterone to dihydrotestosterone occurs to a lesser extent, and conversion to oestradiol is the predominant pathway. Androgen receptors (ARs) have been characterised on immune cells, including neutrophils, macrophages, as well as B and T lymphocytes. Androgens have been shown to affect the functioning of neutrophils, and have broad suppressive effects on the activation of B- and T-cells, however, the molecular mechanisms remain incompletely understood.40

Innate immune regulation mediated by sex hormones

DCs are crucial for the activation of T-cells, and orchestrate immune responses to both self and non-self-antigens. Plasmacytoid dendritic cells (pDCs) express high levels of TLR7 and 9, and are specialised in producing large amounts of type I IFN following viral detection. There are also sex-based differences in pDC function; cis females have been shown to produce higher IFNα levels compared with pDCs from cis males following TLR stimulation. Studies in murine models found this to be dependent on ERα signalling in the pDC. The relative expression levels of ERα and ERβ may hence be important for regulating inflammatory responses. This is further exemplified by ERα/ERβ knockout experiments in SLE-prone murine models, where ERα appeared to protect male mice from disease development. Findings from these studies have been corroborated in humans as lower ERα expression was observed in people with SLE compared with healthy controls.

Sex hormones modulate adaptive immune function

B cells are responsible for the antibody-mediated immune responses including autoreactive responses. Sex hormones have been shown to affect B cell function in vivo. Oestrogen protects autoreactive B cell populations from negative selection and this is thought to contribute to autoimmunity. Oestrogen also promotes antibody production through Th2 polarisation at high oestrogen concentrations. This is supported by detection of elevated serum immunoglobulin (Ig) A and IgG coinciding with an increase in oestrogen concentration before ovulation. Moreover, oestrogen between periovulatory and pregnancy levels appears stimulatory to antibody production,
but inhibitory to the production of B cell precursors between the pro-B to pre-B stages.\textsuperscript{19,44}

In general, cis males have a lower average number of circulating T lymphocytes compared with cis females; potentially due to increased testosterone-mediated T-cell apoptosis in males.\textsuperscript{40,53} This finding is supported by studies of hypogonadal cis males where testosterone supplementation decreased the number of peripheral T-cells, and the relative proportion of regulatory T-cells (Tregs) increased.\textsuperscript{36} The effects of oestrogen on T-cell development and function is more complex, and from previous studies appear to be dose-dependent.\textsuperscript{54–57} The polarisation of naïve CD4\textsuperscript{+} T-cells is essential for effective adaptive immune responses. Th1-polarised T-cells drive cell-mediated immunity towards intracellular pathogens, and Th2-polarised cells induce antibody-mediated immunity.\textsuperscript{57,58} Naïve T-cells may also be polarised to suppressive Treg lineages;\textsuperscript{15} as well as Th9 and Th17 lineages that have both been related to immune-mediated disease.\textsuperscript{59–63} Cytokines produced by Th1- and Th2-polarised cells reciprocally inhibit each other and a failure to produce a sufficiently polarised T helper cell immune response can result in immune pathology, such as autoimmunity or allergy.\textsuperscript{64}

Impact of the X chromosome on immune function

The X chromosome encodes important immune-associated genes, including the AR and TLR7.\textsuperscript{56,65,66} Since cis males have only one copy of the X chromosome, they are more sensitive to mutations in X-linked genes.\textsuperscript{67} In cis females, up to one full X chromosome undergoes transcriptional inactivation in each cell to compensate for gene dosage.\textsuperscript{14} Expression analyses of human genes demonstrate that approximately 15\% of X-linked genes consistently escape X-inactivation silencing, resulting in variable biallelic expression.\textsuperscript{14} It has been suggested that variation in the expression levels of these X-linked genes in cis females may contribute to autoimmune conditions, such as SLE, however, research in this area is conflicting.\textsuperscript{11,16}

Individuals with variations in the number of X chromosomes provide insights into the contribution of X chromosomes and gene dosage on the development of autoimmune disease. Cis males with Klinefelter syndrome have the genotype XXY, and have an increased risk of SLE development compared with XY cis males.\textsuperscript{68} There is a limited body of research that suggests that the Y chromosome exerts changes on the CD4\textsuperscript{+} T-cell transcriptome, which may translate to altered T-cell activation.\textsuperscript{69} This may have relevance for individuals with variation in Y chromosomes,\textsuperscript{69} however, more research is required to validate this theory.

Conversely, cis females with Turner syndrome who are genetically XO may be at an increased risk of developing autoimmune diseases.\textsuperscript{68,70} This is supported by a cohort study of patients with Turner syndrome, which found that 34.4\% of patients were diagnosed with one autoimmune disorder.\textsuperscript{71} The most commonly diagnosed condition in Turner syndrome patients was general thyroid autoimmunity (29.9\%), with 14.9\% diagnosed with Hashimoto’s thyroiditis.\textsuperscript{71} It is hypothesised that the haploinsufficiency of X-linked genes that affect Treg development, such as FOXP3, may prevent Tregs from being able to suppress autoimmune reactions.\textsuperscript{71} Moreover, X-linked genes may contribute to the lack of self-antigen exposure in the thymus, contributing to the pathogenesis of autoimmunity.\textsuperscript{71,72}

Sex-specific immune modulation likely involves the contribution of both sex hormones and X-linked genes. However, it is difficult to determine the relative effects of these biological variables in cis people. Trans people taking sex hormone treatment uncouple these biological variables, and therefore provide important insights into the effects of sex hormones and X-linked genes. A recent study collected peripheral blood mononuclear cells (PBMCs) from a small cohort of trans volunteers and young cis females with Turner syndrome.\textsuperscript{68} From an \textit{in vitro} assay, their cells were treated with a TLR7/8 agonist, and the resulting production of type I IFN was determined. The results of this experiment suggested that TLR7/8-mediated type I IFN production was dependent on the number of X chromosomes and linked to serum testosterone concentrations.\textsuperscript{68} These are important first steps towards understanding immune regulation in trans people.
**Translation of immunological impact of sex hormones**

**Sex hormones impact risk and pathology of autoimmune disease**

Autoimmune diseases are complex and multifactorial in origin, influenced by environmental and genetic factors, hormonal makeup and individual immune system abnormalities. The increased incidence of autoimmunity in cis females is mainly observed following puberty, suggesting that female sex hormones or relative low levels of testosterone may contribute to the sex bias. The ability of B and T-cells to recognise antigens is crucial for protection against pathogens; however, when these cells react to self-antigens, immune pathology can arise. Both B- and T-cells express receptors for sex hormones, and the specific interaction between these cell subsets is related to the pathogenesis of autoimmune diseases.

Sex hormones modulate the maturation of T-cells. The thymus is the key site for the development of a diverse repertoire of T-cells and maintaining central tolerance. In the thymus, expression of self-antigens is regulated by the autoimmune regulator gene (AIRE), which promotes expression of self-antigens resulting in negative selection of self-reactive T-cells. There is increasing evidence that AIRE expression is modulated by sex hormones; testosterone has been identified to upregulate AIRE, whereas oestrogen has been shown to decrease AIRE expression, reducing the efficiency of thymic self-tolerance; this may be a central mechanism driving the increased incidence of autoimmunity in cis females.

Tregs are immune regulatory cells that are crucial for maintaining the balance between effective immune activation and self-tolerance. Decreased numbers of functional Tregs lead to failed self-tolerance and autoimmunity. The number of Tregs increases in the presence of testosterone in vitro, and cis males generally have higher circulating Treg levels compared with cis females. The X-linked transcription factor FoxP3 is responsible for the differentiation and regulatory programme in Tregs; FoxP3 expression can be modulated by testosterone via direct AR binding to FoxP3 gene regulatory sequences. This may be related to the increased number of FoxP3+ Treg cells following testosterone treatment. FoxP3 expression is also regulated by oestrogen. Combined with disease-specific immune regulation, the impact of sex hormones on autoimmune disease is therefore likely significant.

**Systemic lupus erythematosus**

SLE carries one of the strongest sex biases of autoimmune diseases, and is between 7 and 10 times more common in cis females. SLE pathogenesis is heterogeneous and patients often display anti-nuclear antibodies against double-stranded DNA and histones. Anti-nuclear antibodies are considered a hallmark of SLE as they are detectable in over 97% of cases. However, anti-nuclear antibodies are not specific for SLE, and are also detectable in approximately 30% of healthy individuals. SLE pathogenesis has been described as a dysregulated virus response where B cells and pDC are hyper-responsive to TLR7 and/or TLR9 stimulation, resulting in aberrant type I IFN production. As previously discussed, this pathway is susceptible to both sex-specific X chromosomal and hormone influence related to TLR7 gene dosing, and oestrosten- mediated regulation of type I IFN production in pDC. There is also clinical evidence that oestrogen contributes to SLE severity: a meta-analysis of SLE patients found a 25% increase in SLE ‘flares’ during pregnancy. Moreover, the risk of developing SLE appears to be increased in cis females taking oestrogen-containing oral contraceptives and hormone replacement therapy. Conversely, studies of oestrogen deprivation found a reduction in disease severity in cis female SLE patients. The incidence of SLE also decreases sharply after menopause and is uncommon before the onset of puberty. There are also several case reports that document spontaneous SLE in trans females following long-term oestrogen therapy. Together these findings suggest that oestrogen-based gender-affirming therapy may contribute to the development of SLE in genetically susceptible individuals.

**Systemic sclerosis**

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disorder. SSc is more common in cis females compared with cis males, however, cis males experience more severe disease. SSc is characterised by vasculopathy, inflammation and excessive collagen production in the skin and organs. Oestrogen and androgen concentrations have been related to the clinical phenotype of SSc; however, the literature has conflicting findings, and the relationship remains
Incompletely understood. In cis male and female SSc patients, lower concentrations of androgens have been observed compared with healthy controls. A recent study described three cases of trans females developing SSc after long-term oestrogen use. Together, these findings suggest that oestrogen or the reduction of androgens may increase the risk of SSc development.

Multiple sclerosis

MS is a Th1-mediated autoimmune disease that causes chronic inflammation within the central nervous system. Neuroinflammation coupled with relapsing–remitting attacks of demyelination and axonal damage, results in progressive neurological deficits and disability. MS is two to three times more common in cis females; however, cis males with MS tend to experience a more severe disease phenotype and generally have a worse recovery from relapses. Multiple studies have reported that up to 40% of cis males with MS display abnormally low levels of testosterone, compared with controls. Low testosterone has been correlated with increased disability scores, and recent studies have trialled the use of oestrogen as a therapeutic for MS with varied outcomes. Given that testosterone can cross the blood–brain barrier and acts directly on neurons, it is possible that testosterone may be neuroprotective in MS. However, more research is required to validate these findings and rule out confounders, since hypogonadism can be induced by chronic disease states.

In contrast to other autoimmune diseases, oestrogen appears to be ameliorating in MS. For pregnant people with MS, it is well established that there is a strong decline in the risk of MS relapses in the third trimester, which coincides with the highest physiological oestrogen concentrations. However, after delivery when oestrogen levels decline, post-partum relapses are relatively common. Therefore, the hormonal shifts associated with pregnancy may promote an increase in anti-inflammatory Th2 responses. This is supported by a small clinical trial of cis females with MS, which found that supplementation of oestradiol at levels consistent with pregnancy reduced the number of gadolinium-enhancing lesions. However, it is important to consider that there are several important immunological adaptations that occur during pregnancy to prevent rejection of the foetus, and more research in this area is required.

The increased cis female incidence of MS, may also be related to the impact oestrogen has on responses to Epstein–Barr virus (EBV) as recently reviewed. In patients with relapsing–remitting type MS, the clinical symptoms are often associated with a comorbid chronic or recurrent EBV infection, and the presence of EBV-infected B cells in the central nervous system.

A hospital-based study investigated the incidence of MS in trans males and trans females, compared with cis males and cis females. Between 1999 and 2011, this study included 1157 trans females and 2390 trans males; with 4.6 million cis males and 3.4 million cis females in the reference cohorts. From their results, trans females had an increased risk of developing MS with a risk ratio of 6.63 compared with cis females (four observed MS cases and 0.6 expected MS cases). Trans males displayed a risk ratio of 1.44 compared with cis males (five observed MS cases and 3.5 expected MS cases). This suggests that altered levels of both oestrogen and testosterone may contribute to development and/or disease activity in MS.

Trans populations may have higher rates of anti-nuclear antibodies compared with cis populations

The detection of autoimmune disease in a preclinical stage is important for preventing immune-mediated organ damage. However, many autoimmune diseases have similar and overlapping symptoms, which make early and accurate diagnosis challenging. Therefore, it is important to investigate potential novel diagnostic biomarkers. As previously discussed, anti-nuclear antibodies may precede development of autoimmunity; a recent study observed that 36% of trans males and 31% of trans females were positive for anti-nuclear antibodies, compared with 13% in the cis male/female population, which was lower than previously reported elsewhere. Since the trans male/female groups consistently reported higher positivity for anti-nuclear antibodies compared with the cis group, this indicates that both oestrogen and testosterone may modulate endogenous control mechanisms. In summary, although autoimmune diseases are rare, given the contributing effects of oestrogen to disease development, they may pose a small but significant risk in genetically susceptible trans individuals, particularly to those on oestrogen treatment.
The role of sex hormones in allergic diseases

Allergic disease is the clinical manifestation of atopic and IgE-sensitised individuals, where exposure to exogenous antigens results in acute inflammation. Allergic reactions are mediated by mast cell degranulation via IgE-allergen complexes, inflammatory mediators and pro-inflammatory cytokines. There is a disproportionate representation of atopy and allergic disease in cis males before puberty and cis females after puberty. It is well reported that cis male children exhibit higher rates of allergic asthma compared with cis females before puberty; however, following puberty, asthma prevalence and severity increases in cis females, and conversely decreases in cis males. This suggests that testosterone may be protective in allergic disease, whereas oestrogen may aggravate allergic diseases, such as asthma. In non-pregnant cis females, the levels of sex hormones fluctuate significantly throughout the menstrual cycle. These are associated with variability in allergic reactions, and may influence the outcome of skin prick test results.

Trans individuals experience significant socio-economic disparities, which may predispose to higher rates of chronic conditions, including asthma and allergic disease, compared with cis individuals. There is a case report describing one trans female with significant past medical history of allergies and asthma; subsequently upon initiation of oestrogen-based gender-affirming therapy, she experienced an increase in allergic manifestations and increased asthma exacerbations. It is well documented that trans people face disproportionate barriers to accessing health care compared with cis people. Since it is currently not commonplace for medical practitioners to receive training on trans health, many clinicians are often uninformed of a trans person’s specific needs and unable to provide appropriate recommendations for their care. As a result, trans people are more likely to delay seeking medical attention, resulting in increased morbidity from poor asthma control, for example. Asthma management in trans people may also be complicated by chest binding (to minimise breast appearance) which may result in symptoms that mimic or exacerbate asthma. As sex hormones have a demonstrated effect on allergic disease, this is an important issue to consider, particularly for atopic individuals as they initiate gender-affirming hormone therapy. Medical treatment for atopic disease may need to be adjusted as hormone levels change, to better manage atopic exacerbations in trans individuals.

Sex hormones impact cancer development and efficiency of immunotherapy

It is well established that cis males are more susceptible to developing cancer and haematological malignancies: the lifetime risk of developing cancer is one in two for cis males, and one in three for cis females. Moreover, with the average lifetime risk of dying of cancer being one in three cis males and one in six cis females, cis males also experience a disproportionately high mortality from cancer. Immune surveillance is crucial in preventing the development and progression of cancer and as mentioned above, this is strongly impacted by sex hormones. Moreover, in some cancers, such as breast or prostate cancer, sex hormones directly impact pathogenesis; and therefore, gonadotropin-releasing hormone (GnRH) agonist drugs are routinely used to suppress the production of sex hormones. However, there are also sex differences in the incidence of other cancers at both genetic and molecular levels, and many cancers have been shown to be both positively or negatively affected by sex hormones. An examination of cancer incidence across age groups found that cis male children also had an increased susceptibility to cancers, suggesting that not only sex hormones contribute to the discrepancy in incidence between cis males and cis females.

The ability of T-cells to recognise tumour-associated antigens is an important function of immune surveillance. In particular, CD8+ cytotoxic T-cells secrete cytokines, such as IFNγ and TNF to produce potent antitumour immunity. CD4+ T-cells have also been shown to improve tumour clearance through recognition of tumour neo-antigens, and an ability to modulate the tumour microenvironment. However, cancer cells have the ability to modify the expression of their surface molecules and key antigens, to evade the immune response and foster an environment that supports the growth of a malignancy. Interestingly, some of these pathways including the function of Tregs appear to be sex-specific.

Immunotherapeutic agents are used to increase the activation of the immune system to fight cancer cells through cell-mediated mechanisms.
These have improved treatment efficacy and reduced side effects compared with traditional treatments, which are toxic and have limited specificity.123 Sex hormones have been identified as drivers of sex-based differences in immunotherapy outcomes.123 In particular, oestrogen appears to modulate the efficiency of immunotherapy; therefore, it is crucial to further investigate the role of sex hormones in non-reproductive cancers, to maximise the effectiveness of treatment.123 Immune checkpoint inhibitors have had remarkable success in patients with metastatic disease; however, there are also a large number of patients that do not respond to treatment, or develop toxicities (immune-related adverse events).125 A small number of studies suggest that cis female cancer patients experience lower rates of treatment success from checkpoint inhibitors.125,126 Since immune checkpoints, including the programmed death protein/ligand (PD-1/PD-L1) pathway, are modulated upstream by oestrogen, sex hormones may influence the sex disparity in treatment success.127 There are also important sex-based differences in terms of local tumour invasion and metastatic spread that warrants further research.128

Melanoma was previously considered a non-hormone-related cancer, however, epidemiological data strongly suggest that cis females have a survival advantage compared with cis males.128 Moreover, following menopause declining oestrogen levels were correlated with a reduction in survival in cis females.128 There is also increasing evidence that the disruption of oestrogen signalling via ERα and ERβ is related to cancer initiation.128 This is supported by findings from clinical trials using ER antagonists including tamoxifen in combination with standard chemotherapy. When bound to ERα, tamoxifen has been shown to decrease cell proliferation; this is supported by a meta-analysis investigating the use of tamoxifen in advanced melanoma.128 From nine randomised control trials, it was found that patients treated with tamoxifen were ‘more likely to respond to chemotherapy’. However, since these trials exclusively recruited patients with advanced melanoma, there were overall no differences in mortality.129

A review, investigating primary care in trans people, suggests that cancer is the least researched aspect of the global burden of disease in trans individuals.130 However, a recent cohort study investigated the incidence of breast cancer in cis and trans individuals in the Netherlands.131 This study determined that although breast cancer is rare in cis males, trans females taking gender-affirming hormonal therapy were at an increased risk of invasive breast cancer. Contrastingly, trans males taking gender-affirming hormones were at a decreased risk of breast cancer compared with cis females.131 In this cohort, most of the tumours detected were oestrogen and progesterone receptor positive; and the breast cancer found in trans females was phenotypically similar to that detected in cis females.131 Moreover, the observed decreased risk of breast cancer in trans males may also be attributed to some participants having had a gender-affirming mastectomy (top surgery). It is important to note that the absolute risk of breast cancer in trans people is low, and from this study the incidence of breast cancer in trans males and females was lower than the incidence in cis females.131 Moreover, gender-affirming hormonal therapy has been shown to be safe and effective.131 However, as it is established that oestrogen and testosterone contribute to development of hormone-sensitive reproductive cancers, including breast and prostate cancer in cis people;132 it may be beneficial for trans people taking oestrogen or testosterone to be proactively screened for breast and prostate cancer, especially for those with a family history or genetic susceptibility.

Sex hormones influence the immune response to infectious disease

Sex-based immune differences impact susceptibility to bacterial and viral pathogens.133,134 Effective defence against infectious diseases requires the coordination between the innate and adaptive arm of the immune system.24 Following the detection of an infectious agent, the innate immune response initiates a series of non-specific reactions, initiated through highly conserved pattern recognition receptors (PRRs), such as TLRs.135 PRRs recognise molecular motifs on pathogens, such as lipopolysaccharide on gram-negative bacteria.136 Following the innate response, the adaptive immune system generates highly specific responses to defend the host against pathogens. The coordination between B- and T-cell subsets is vital in the production of protective immunity from infectious diseases, to produce long-lasting immunological memory.136,137

In general, cis males experience a higher incidence of infectious diseases compared with cis
females, which has previously been attributed to weaker innate immune responses. Some of this may be due to the variability in expression of the TLRs involved in viral recognition, such as TLR7 and 8. Like TLR7, TLR8 is expressed on the X chromosome, and performs a key role in anti-viral immunity. Viruses can induce inhibitory pathways mediated by interleukin 10 (IL-10) to evade immune surveillance, and successfully infect the host. Increased IL-10 production has the effect of preventing the maturation of DCs, and activating suppressive immune cells, such as Tregs. From an in vitro study, following influenza infection cis male PBMCs had a four-fold higher concentration of IL-10 compared with cis females, whereas, cis females instead produced elevated levels of type I IFN. HIV is an infectious disease where early intervention and management is crucial for preventing long-term morbidity and mortality from AIDS progression. In general, cis female patients with HIV have a better prognosis than cis males. It is theorised that sex hormones impact early infection viral load, and oestrogen may modulate the production of the HIV-1 reservoir, resulting in better disease outcomes in cis females. HIV-1 single stranded RNA (ssRNA) is recognised by TLR7 on pDCs, and as previously discussed TLR7 is differentially expressed in cis males and cis females resulting in significant sexual dimorphism. Experiments of HIV-1 mediated TLR7 stimulation resulted in a significantly higher number of IFNα producing pDCs in cis females compared with cis males. Therefore, the anti-viral functions of IFN stimulated genes may contribute to the higher immune activation of CD4+ and CD8+ T-cells during persistent HIV-1 infection in cis females. In additional studies, cis females displayed higher HIV-1 transcription suppression in response to oestradiol, and subsequently higher reactivity following oestrogen receptor 1 (ESR-1) modulation. Together, these findings suggest that ESR-1 is a critical regulator of HIV-1 latency.

Bacterial infections appear to be more common in cis males who experience a higher incidence of gastrointestinal and respiratory bacterial diseases and sepsis compared with cis females, whereas cis females are more prone to genitourinary tract bacterial infections. This discrepancy has been long established, with studies from the late 60s observing that the mortality rate following Mycobacterium tuberculosis infection was lower in gonadectomised cis males compared with cis male healthy controls. Contrastingly, the tuberculosis-associated mortality was 10-fold higher in ovariectomised cis females compared with healthy controls. Together, these findings indicate that sex hormones influence the outcomes of bacterial infections in cis males and cis females. These results are also supported by more recent studies observing an increase in opportunistic infections following menopause in cis females. Some of these effects are mediated directly by oestradiol, but depend on the ER subtype and specific tissue compartment that is involved in the infection. It is also likely that this effect stems from differential responses in macrophages in regards to TLR expression, which appears elevated in cis females compared with cis males. This would allow for an increased capacity to detect and eliminate pathogens.

It is important to note that although a biological difference in immune responses is observed between cis males and cis females, different susceptibility to infections may also be a result of behavioural and environmental factors that contribute to exposure. Epidemiological data found that infections with Dengue, Hantaviruses and Hepatitis B and C are more common in cis males than cis females, and this may be explained by behavioural differences that cause higher exposure rates in cis males. There are also other demographic variables, such as obesity, which are associated with impaired anti-viral immunity. Published literature on the susceptibility to infectious disease between the sexes consistently fails to recognise the intersection with gender. Therefore, it is unclear how to extrapolate cisgender sex-based findings in the context of infection susceptibility in trans people. Currently, there is little data regarding the broad incidence of infectious disease in trans people, however, it has been reported that trans people have a higher incidence of HIV and sexually transmitted infections (STIs), particularly in trans females compared with the cis population. Trans people have often experienced discrimination, stigma and health care provider ignorance in previous attempts to access medical care, which often results in hesitancy to seek out medical attention, which delays treatment. These barriers have been reported to result in less frequent HIV/STI testing. In an Australian national survey of sexual health clinics, it was found that the incidence of HIV was around...
3.5% in trans males and 5.7% in trans females on first visit, which is significantly higher than 1.2% in cis individuals. Given the role of sex hormones and the concerning finding of increased HIV prevalence in the trans population, this is an area that requires further research.

**Sex hormones impact responses to COVID-19**

With the emergence of COVID-19, there has been a renewed focus on sex-specific virus-induced morbidity. As with the related severe acute respiratory syndrome (SARS) virus, the SARS-CoV-2 virus also displays a disproportionately higher morbidity in cis males compared with cis females. COVID-19 is a highly heterogeneous viral illness with clinical severity ranging from a mild self-limiting respiratory syndrome, to multi-organ failure and cytokine storm that is associated with a high mortality rate. Case data from Europe suggest that the cis male to cis female ratio of hospitalisations is around 1.5:1, with case fatality at 1.8:1 in unvaccinated individuals. There is also an exponential relationship between age and infection mortality, starting at 0.01% at age 25 and increasing to 15% at age 85. SARS-CoV-2 is an ssRNA virus, therefore infection by this virus stimulates TLR7 and produces type I IFN early in the infection cascade. It is thought that severe COVID-19 disease may be related to a failure in early activation of IFN signalling in cis males. This is exemplified in a case study of four severe COVID-19 cis male patients, which identified loss of function variants in TLR7 in all four patients. Another study of people with severe COVID-19 identified that IFN deficiency, and the presence of anti-IFN antibodies were all related to worse clinical outcomes.

Although it is established that cis males are at an increased risk of severe COVID compared with cis females, there is little data on how COVID-19 morbidity/mortality affects trans individuals. As severe COVID-19 infections have been linked to early TLR failure in cis males, the variable bi-allelic expression of TLRs in trans males may offer more protection compared with cis males, however, more research in this area is required. From available data, it is clear that COVID-19 has disproportionately impacted trans individuals’ access to care; with gender services being labelled as ‘non-essential’ at many facilities, trans individuals have faced new barriers to receiving their regular care. Despite the recent research efforts to categorise sex-based differences in the disease progression and outcomes of COVID-19, only a minority of countries/states report on COVID infections and mortality with more than two options for gender, and trans identity is not indicated in the data. The direct impact of COVID infection on trans individuals therefore remains largely unknown.

**Sex hormones impact responses to vaccines**

Vaccines are an important tool in preventing infectious disease. However, historically cis males and cis male-derived cells have been predominantly used for human health research. Furthermore, until the 1990s, cis females of ‘childbearing’ age were routinely excluded from drug trials, since fluctuating levels of hormones were considered an undesirable extraneous biological variable. This has resulted in an under-appreciation of the effect of biological sex on vaccine responses. There is an accumulating body of research that demonstrates biological sex is an important predictor of immunisation efficacy: cis females overall display higher antibody levels and T-cell activation following vaccination. However, cis females also experience more frequent and severe adverse reactions. The measles–mumps–rubella (MMR) vaccine consists of three strains of attenuated virus, and is routinely given to infants. A cross-sectional study of children over 15 years of age found that following vaccination, the rate of seroconversion was higher in cis females compared with cis males. Another study of people with severe COVID-19 identified that IFN deficiency, and the presence of anti-IFN antibodies were all related to worse clinical outcomes.

The measles–mumps–rubella (MMR) vaccine consists of three strains of attenuated virus, and is routinely given to infants. A cross-sectional study of children over 15 years of age found that following vaccination, the rate of seroconversion was higher in cis females compared with cis males. Regression modelling indicated that time of vaccination and being female were the two most important predictors for antibody persistence following vaccination. Interestingly, a study of pre-pubertal children found that cis females only had a transiently higher level of anti-rubella antibodies 2–4 weeks post-vaccination compared with cis males, and this sex difference was not apparent at a 10-week follow-up. Following this, in post-pubertal adolescents (14–17 years of age follow-up), cis females consistently displayed higher anti-rubella IgG titres than cis males of the same age. This observation was not only specific for rubella, but was also observed following influenza vaccination; adult cis females generated antibody titres twice as high as cis males when administered a half or full dose of an inactivated influenza vaccine. Moreover, studies of the pandemic H1N1 vaccine demonstrated...
that cis females produced higher amounts of IL-6 and antibody responses than adult cis males. Together, these findings suggest that the onset of puberty and rise in sex hormone concentration is related to the observable sexual dimorphism in vaccine-induced antibody titres.

In the age of the COVID-19 pandemic, it is also important to consider sex-based differences in responses to COVID-19 vaccinations. In Australia, over 58 million vaccine doses have been administered to date, and >95% of people over the age of 16 are fully vaccinated. There is a small amount of sex-disaggregated data on COVID-19 adverse reactions, indicating that cis females account for a higher proportion of adverse reactions (70.9–76%) when compared with cis males (22.4–28.6%). This is consistent with previous findings on vaccinations. However, so far, there is little data available on rates of COVID-19 vaccination and outcomes in trans people.

Transgender young people

There is an increasing appreciation in the scientific community that sex hormones are important for immune function, as summarised in Table 1. Despite this, there is relatively little research regarding the immune function of trans people undertaking gender-affirming hormonal therapy. Moreover, there are currently no long-term studies on the health outcomes of trans young people. From a 2012 population-based study in New Zealand, the number of trans young people was estimated by self-report to be around 1.2% of adolescents. More recently, a 2019 study of high-school-aged young people in Australia indicated that 2.3% identified as trans and/or gender diverse. This trend also reflects an increase of referrals for specialist gender-affirming care in recent years. However, some, but not all trans young people seek gender-affirming medical treatment.

Gender-affirming medical care in trans young people depends on the age of the young person, stage of biologically determined pubertal development (described as ‘Tanner staging of puberty’), the young person’s wishes, parental consent and the young person’s capacity to consent. Some young transgender adolescents seek GnRH agonist treatment in the early stages of puberty to suppress further pubertal development. Following a period of puberty suppression, the trans young person as an older adolescent may wish to commence treatment with gender-affirming hormones, including oestrogen or testosterone. This has the effect of inducing secondary sexual characteristics that align with their gender identity, and in Australia this treatment may commence with parental consent, over the age of 16 or when the young person is found to have mature capacity to consent to this treatment. Older adolescents may present after puberty is complete, seeking oestrogen or testosterone treatment without preceding puberty suppression. For young people who have significant gender dysphoria that request gender-affirming treatment, these treatments are considered potentially lifesaving and beneficial to quality of life. This is supported by national and international guidelines, standards of care, and an increasing number of observational studies.

In studies of cis populations, the onset of puberty has been linked to an increase in atopic disorders and autoimmunity in cis females, this suggests a mechanistic relationship between an increase in oestrogen and the development of an immune-mediated disorder in genetically predisposed individuals. The impact of gender-affirming puberty suppression, anti-androgen treatment, oestrogen and testosterone therapy on immune system function in trans adolescents is currently unknown. Therefore, it is crucial that we have a more comprehensive understanding of the potential effects of these treatments, so that trans young people can make informed decisions regarding their own health care, and health care providers can tailor care to maximise wellbeing.

Conclusion

There is an increasing body of research aiming to understand the mechanisms by which the immune system differs in cis males and cis females. The immunological impact of sex hormones results in different disease susceptibility between the sexes, with relevance across a range of human diseases. Given the immune-activating properties of oestrogen, it seems likely that oestrogen treatment may induce a lower threshold for immune activation in trans females. Therefore, trans females with a genetic susceptibility may be at an increased risk of developing disorders of immune hyperactivation, such as autoimmunity and allergy. However, they may at the same time be at reduced risk of developing cancers due to more efficient immune surveillance. As cis males produce...
Table 1. A summary of the effects of oestrogen and testosterone on immune function. The impacts of these hormones are well documented in cisgender (cis) males and cis females, however, the potential effects of sex hormones on immune function in transgender (trans) people is relatively unknown.

| Immune pathology                      | Oestrogen                                                                 | Testosterone                                                                 |
|----------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Autoimmune disease (general)           | Protects autoreactive B cells from negative selection, at high-dose oestrogen causes involution of the thymus | Affects the development of Tregs, in cis males, there is a greater number of Tregs compared with peripheral T cells |
|                                       | Reduces AIRE expression, reducing thymic self-tolerance                   | Increases AIRE expression, promoting thymic self-tolerance                   |
| Autoimmune disease (MS)               | High-dose oestrogen ameliorates MS flares during pregnancy in cis females | Testosterone can cross the blood-brain barrier and may be neuroprotective in MS |
| Autoimmune disease (SLE)              | High-dose oestrogen aggravates SLE, increased flares observed during pregnancy in cis females | Reduced testosterone is related to an increase in autoimmune diseases, such as SLE in cis males |
| Allergic disease                       | The variability of hormones including oestrogen during the menstrual cycle in cis females influences allergic reactions, may affect the outcome of skin prick tests | Cis male children exhibit higher rates of allergic asthma pre puberty. After puberty, allergic asthma incidence is lower in cis males compared with cis females, suggesting testosterone may be protective |
| Cancer – immunotherapy                | Immune checkpoint inhibitors, such as PD-1/PD-L1 are modulated upstream by oestrogen signalling. Higher oestrogen levels are associated with a survival advantage in cis female melanoma patients | Cis males experience a disproportionate burden of malignancies. Immune surveillance may be less effective in cis males due to testosterone-mediated immune suppression |
| Infectious disease                    | TLR7 is an X-linked viral sensor, induces inflammatory type I IFN and is more highly expressed in cis females. Oestrogen regulates type I IFN production from plasmacytoid dendritic cells following TLR7 signalling | In hypogonadal cis males, decreased testosterone levels correlate with increased inflammatory markers (CRP and TNF). Testosterone-mediated immune suppression may contribute to the increased incidence of sepsis in cis males |
|                                        | ER-mediated TLR7 signalling is crucial for early immune responses to viruses, and may explain the survival advantage of cis females in COVID-19 | Neutrophil development is dependent on AR signalling. In rat models, testosterone potentiated neutrophil activation and reduced the survival of neutrophils |
| Vaccine responses                      | Oestrogen, or female sex hormones, likely contribute to efficient vaccine responses. Pre-pubertal cis male and cis female children had similar anti-rubella antibody titres, post-puberty cis females consistently produce higher IgG titres than age-matched cis males | Cis males experience less adverse events and produce less inflammatory cytokines, such as IL-6 following vaccinations. Testosterone may contribute to less protective antibody responses, but more research is required |

AIRE, autoimmune regulator gene; AR, androgen receptor; CRP, C-reactive protein; IFN, interferon; MS, multiple sclerosis; SLE, systemic lupus erythematosus; PD-1, programmed death protein-1; PD-L1, programmed death ligand-1; TLR, toll-like receptor; TNF, tumour necrosis factor.
less-efficient immune responses following vaccination;\textsuperscript{2,3,163} trans males may experience less robust antibody-mediated responses and reduced protection from previous vaccinations. This is of renewed importance in light of the COVID-19 pandemic, where successful seroconversion following vaccination is highly effective in reducing COVID-related morbidity and mortality.\textsuperscript{151} Moreover, the generalised immune suppressive effects of androgens\textsuperscript{6,186} may result in trans males being more prone to infections. Given the clear role of the sex hormones oestrogen and testosterone as immune modulators, there is a need for continued research into the immunological impact of sex hormones, and identification of associated specific health care needs, to ensure the positive long-term health outcomes of trans people.

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All authors have consented for the manuscript to be published.

Author contributions
Alice A. White: Conceptualisation; Investigation; Writing – original draft.
Ashleigh Lin: Supervision; Writing – review & editing.
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Julia K. Moore: Writing – review & editing.
Aris Siafarikas: Writing – review & editing.
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Jonatan Leffler: Conceptualisation; Funding acquisition; Project administration; Supervision; Writing – review & editing.

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References
1. Moulton VR. Sex hormones in acquired immunity and autoimmune disease. \textit{Front Immunol} 2018; 9: 2279.
2. Giefing-Kröll C, Berger P, Lepperdinger G, \textit{et al}. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. \textit{Aging Cell} 2015; 14: 309–321.
3. Flanagan KL, Fink AL, Plebanski M, \textit{et al}. Sex and gender differences in the outcomes of vaccination over the life course. \textit{Annu Rev Cell Dev Biol} 2017; 33: 577–599.
4. Cunningham M and Gilkeson G. Estrogen receptors in immunity and autoimmunity. \textit{Clin Rev Allergy Immunol} 2011; 40: 66–73.
5. Billi AC, Kahlenberg JM and Gudjonsson JE. Sex bias in autoimmunity. \textit{Carr Opin Rheumatol} 2019; 31: 53–61.
6. Trigunaite A, Dimo J and Jørgensen TN. Suppressive effects of androgens on the immune system. \textit{Cell Immunol} 2015; 294: 87–94.
7. Klein SL and Flanagan KL. Sex differences in immune responses. \textit{Nat Rev Immunol} 2016; 16: 626–638.
8. Clocchiatti A, Cora E, Zhang Y, \textit{et al}. Sexual dimorphism in cancer. \textit{Nat Rev Cancer} 2016; 16: 330–339.
9. Cook MB, Dawsey SM, Freedman ND, \textit{et al}. Sex disparities in cancer incidence by period and age. \textit{Cancer Epidemiol Biomarkers Prev} 2009; 18: 1174–1182.
10. Dorak MT and Karpuzoglu E. Gender differences in cancer susceptibility: an inadequately addressed issue. *Front Genet* 2012; 3: 268.

11. Laffont S, Rouquié N, Azar P, *et al.* X-chromosome complement and estrogen receptor signaling independently contribute to the enhanced TLR7-mediated IFN-α production of plasmacytoid dendritic cells from women. *J Immunol* 2014; 193: 5444–5452.

12. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol* 2008; 8: 737–744.

13. Souyris M, Cenac C, Azar P, *et al.* TLR7 escapes X chromosome inactivation in immune cells. *Sci Immunol* 2018; 3: 8855.

14. Berletch JB, Yang F, Xu J, *et al.* Genes that escape from X inactivation. *Hum Genet* 2011; 130: 237–245.

15. Walecki M, Eisel F, Klug J, *et al.* Androgen receptor modulates Foxp3 receptor in CD4+CD25+Foxp3+ regulatory T-cells. *Mol Biol Cell* 2015; 26: 2845–2857.

16. Berghöfer B, Frommer T, Haley G, *et al.* TLR7 ligands induce higher IFN-α production in females. *J Immunol* 2006; 177: 2088–2096.

17. Bhatia A, Sekhon HK and Kaur G. Sex hormones and immune dimorphism. *Sci World J* 2014; 2014: 159150–159158.

18. Brown MA and Su MA. An inconvenient variable: sex hormones and their impact on T cell responses. *J Immunol* 2019; 202: 1927–1933.

19. Cutolo M, Sulli A, Capellino S, *et al.* Sex hormones influence on the immune system: basic and clinical aspects in autoimmunity. *Lupus* 2004; 13: 635–638.

20. Beery AK and Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev* 2011; 35: 565–572.

21. Schul SL. The informed consent model of transgender care: an alternative to the diagnosis of gender dysphoria. *J Humanist Psychol* 2018; 58: 72–92.

22. Gooren LJ, Kreukels B, Lapauw B, *et al.* (Patho) physiology of cross-sex hormone administration to transsexual people: the potential impact of male-female genetic differences. *Andrologia* 2015; 47: 5–19.

23. Warrington R, Watson W, Kim HL, *et al.* An introduction to immunology and immunopathology. *Allerg Asthma Clin Immunol* 2011; 14: 49.

24. Draper CF, Duisters K, Weger B, *et al.* Menstrual cycle rhythmicity: metabolic patterns in healthy women. *Sci Rep* 2018; 8: 14568.

25. Grimaldi CM, Cleary J, Dagtas AS, *et al.* Estrogen alters thresholds for B cell apoptosis and activation. *J Clin Invest* 2002; 109: 1625–1633.

26. Aguilar-Pimentel JA, Cho Y-L, Gerlini R, *et al.* Increased estrogen to androgen ratio enhances immunoglobulin levels and impairs B cell function in male mice. *Sci Rep* 2020; 10: 18334.

27. Khan D and Ansar Ahmed S. The immune system is a natural target for estrogen action: opposing effects of estrogen in two prototypical autoimmune diseases. *Front Immunol* 2016; 6: 635.

28. Sanchez-Guerrero J, Karlson EW, Liang MH, *et al.* Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 804–808.

29. Petri M, Kim MY, Kalunian KC, *et al.* Combined oral contraceptives in women with systemic lupus erythematosus. *NEJM* 2005; 353: 2550–2558.

30. Zen M, Ghirardello A, Iaccarino L, *et al.* Hormones, immune response, and pregnancy in healthy women and SLE patients. *Swiss Med Wkly* 2010; 140: 187–201.

31. Zoller AL and Kersh GJ. Estrogen induces thymic atrophy by eliminating early thymic progenitors and inhibiting proliferation of β-selected thymocytes. *J Immunol* 2006; 176: 7371–7378.

32. Robinson DP and Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. *Horm Behav* 2012; 62: 263–271.

33. Schumacher A, Costa SD and Zenclussen AC. Endocrine factors modulating immune responses in pregnancy. *Front Immunol* 2014; 5: 196.

34. Holst JP, Soldin OP, Guo T, *et al.* Steroid hormones: relevance and measurement in the clinical laboratory. *Clin Lab Med* 2004; 24: 105–118.

35. Ben-Batalla I, Vargas-Delgado ME, von Amsberg G, *et al.* Influence of androgens on immunity to self and foreign: effects on immunity and cancer. *Front Immunol* 2020; 11: 1184.

36. Baillargeon J, Al Snih S, Raji MA, *et al.* Hypogonadism and the risk of rheumatic
autoimmune disease. Clin Rheumatol 2016; 35: 2983–2987.

38. Chitnis T. The role of testosterone in MS risk and course. Mult Scler 2018; 24: 36–41.

39. Fijak M, Schneider E, Klug J, et al. Testosterone replacement effectively inhibits the development of experimental autoimmune orchitis in rats: evidence for a direct role of testosterone on regulatory T cell expansion. J Immunol 2011; 186: 5162–5172.

40. Lai JJ, Lai KP, Zeng W, et al. Androgen receptor influences on body defense system via modulation of innate and adaptive immune systems. Am J Pathol 2012; 181: 1504–1512.

41. Seillet C, Laffont S, Trémollières F, et al. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor α signaling. Blood 2012; 119: 454–464.

42. Tang F, Du Q and Liu YJ. Plasmacytoid dendritic cells in antiviral immunity and autoimmunity. Sci China Life Sci 2010; 53: 172–182.

43. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. Cell Immunol 2015; 294: 63–69.

44. Straub RH. The complex role of estrogens in inflammation. Endocr Rev 2007; 28: 521–574.

45. Walker VR and Korach KS. Estrogen receptor knockout mice as a model for endocrine research. ILAR J 2004; 45: 455–461.

46. Rider V, Li X, Peterson G, et al. Differential expression of estrogen receptors in women with systemic lupus erythematosus. J Rheumatol 2006; 33: 1093–1101.

47. Molloy EJ, O’Neill AJ, Grantham JJ, et al. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. Blood 2003; 102: 2653–2659.

48. Fine N, Tasevski N, McCulloch CA, et al. The neutrophil: constant defender and first responder. Front Immunol 2020; 11: 571085.

49. Deitch EA, AnanthaKrishnan P, Cohen DB, et al. Neutrophil activation is modulated by sex hormones after trauma-hemorrhagic shock and burn injuries. Am J Physiol Heart Circ Physiol 2006; 291: H1456–H1465.

50. Gupta S, Nakabo S, Blanco LP, et al. Sex differences in neutrophil biology modulate response to type I interferons and immunometabolism. PNAS 2020; 117: 16481–16491.

51. Bouman A, Heineman MJ and Faas MM. Sex hormones and the immune response in humans. Hum Reprod Update 2005; 11: 411–423.

52. Sakiani S, Olsen NJ and Kovacs WJ. Gonadal steroids and humoral immunity. Nat Rev Endocrinol 2013; 9: 56–62.

53. McMurray RW, Suwannaroj S, Ndebele K, et al. Differential effects of sex steroids on T and B cells: modulation of cell cycle phase distribution, apoptosis and bel-2 protein levels. Pathobiology 2001; 69: 44–58.

54. Cutolo M, Brizzolara R, Atzeni F, et al. The immunomodulatory effects of estrogens. Ann NY Acad Sci 2010; 1193: 36–42.

55. Burleson MH, Malarkey WB, Cacicoppo JT, et al. Postmenopausal hormone replacement: effects of autonomic, neuroendocrine and immune reactivity to brief psychological stressors. Psychosom Med 1998; 60: 17–25.

56. Pennell LM, Galligan CL and Fish EN. Sex affects immunity. J Autoimmun 2011; 38: 282–291.

57. Bettelli E, Sullivan B, Szabo SJ, et al. Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. Exp Med 2004; 200: 79–87.

58. Mosmann TR, Cherwinski H, Bond MW, et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986; 136: 2348–2357.

59. Kaplan MH. Th9 cells: differentiation and disease. Immunol Rev 2013; 252: 104–115.

60. Schmitt E, Klein M and Bopp T. Th9 cells, new players in adaptive immunity. Trends Immunol 2014; 35: 61–68.

61. Goswami R and Kaplan MH. A Brief History of IL-9. J Immunol 2011; 186: 3283–3288.

62. Steinman L. A brief history of TH17, the first major revision in the TH1/TH2 hypothesis of T cell–mediated tissue damage. Nat Med 2007; 13: 139–145.

63. Tesmer LA, Lundy SK, Sarkar S, et al. Th17 cells in human disease. Immunol Rev 2008; 223: 87–113.

64. Rubtsova K, Marrack P and Rubtsov AV. TLR7, IFNγ, and T-bet: their roles in the development of ABCs in female-biased autoimmunity. Cell Immunol 2015; 294: 80–83.

65. Umiker BR, Andersson S, Fernandez L, et al. Dosage of X-linked Toll-like receptor 8 determines gender differences in the development
of systemic lupus erythematosus. *Eur J Immunol* 2014; 44: 1503–1516.

66. Davey RA and Grossmann M. Androgen receptor structure, function and biology: from bench to bedside. *Clin Biochem Rev* 2016; 37: 3–15.

67. Cunningham RL, Lumia AR and McGinnis MY. Androgen receptors, sex behaviour, and aggression. *Neuroendocrinol* 2012; 96: 131–140.

68. Webb K, Peckham H, Radziszewska A, et al. Sex and pubertal differences in the type 1 interferon pathway associate with both X chromosome number and serum sex hormone concentration. *Front Immunol* 2019; 9: 3167.

69. Case LK, Wall EH, Dragon JA, et al. The Y chromosome as a regulatory element shaping immune cell transcriptomes and susceptibility to autoimmune disease. *Genome Res* 2013; 23: 1474–1485.

70. Thrasher BJ, Hong LK, Whitmire JK, et al. Epigenetic dysfunction in turner syndrome immune cells. *Curr Allergy Asthma Rep* 2016; 16: 36.

71. Wegiel M, Antosz A, Gieburowska J, et al. Autoimmunity predisposition in girls with turner syndrome. *Front Endocrinol* 2019; 10: 511.

72. Bianchi I, Lleo A, Gershwin ME, et al. The X chromosome and immune associated genes. *J Autoimmun* 2012; 38: 187–192.

73. Nussinovitch U and Shoenfeld Y. The role of gender and organ specific autoimmunity. *Autoimmun Rev* 2011; 11: 377–385.

74. Kisiel BM, Kosinska J, Wierzbowska M, et al. Differential association of juvenile and adult systemic lupus erythematosus with genetic variants of oestrogen receptors alpha and beta. *Lupus* 2011; 20: 85–89.

75. Petersone L, Edner NM, Ovcinnikovs V, et al. T cell/B cell collaboration and autoimmunity: an intimate relationship. *Front Immunol* 2018; 9: 1941.

76. Bakhru P and Su MA. Estrogen turns down ‘the AIRE’. *J Clin Investig* 2016; 126: 1239–1241.

77. Dragan N, Bismuth J, Cizeron-Clairac G, et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J Clin Investig* 2016; 126: 1525–1537.

78. Lasrada N, Jia T, Massilamany C, et al. Mechanisms of sex hormones in autoimmunity: focus on EAE. *Biol Sex Differ* 2020; 11: 50.

79. Tan EM, Feltkamp TE, Smolen JS, et al. Range of antinuclear antibodies in 'healthy' individuals. *Arthritis Rheum* 1997; 40: 1601–1611.

80. Petes C, Odoardi N and Gee K. The toll for trafficking: toll-like receptor 7 delivery to the endosome. *Front Immunol* 2017; 8: 1075.

81. Taneja V. Sex hormones determine immune response. *Front Immunol* 2018; 9: 1931.

82. Lefèvre N, Corazza F, Valsamis J, et al. The number of X chromosomes influences inflammatory cytokine production following toll-like receptor stimulation. *Front Immunol* 2019; 10: 1052.

83. Smyth A, Oliveira GH, Lahd BD, et al. A systematic review and meta-analysis of pregnancy outcomes in patients with systemic lupus erythematosus and lupus nephritis. *Clin J Am Soc Nephrol* 2010; 5: 2060–2068.

84. Chan KL and Mok CC. Development of systemic lupus erythematosus in a male-to-female transsexual: the role of sex hormones revisited. *Lupus* 2013; 22: 1399–1402.

85. Desai MK and Brinton RD. Autoimmune disease in women: endocrine transition and risk across the lifespan. *Front Endocrinol (Lausanne)* 2019; 10: 265.

86. Hill BG, Hodge B and Misischia R. Lupus nephritis in a transgender woman on cross-sex hormone therapy: a case for the role of oestrogen in systemic lupus erythematosus. *Lupus* 2020; 29: 1807–1810.

87. Campochiaro C, Host LV, Ong VH, et al. Development of systemic sclerosis in transgender females: a case series and review of the literature. *Clin Exp Rheumatol* 2018; 36(Suppl. 113): 50–52.

88. Hughes M, Pauling JD, Armstrong-James L, et al. Gender-related differences in systemic sclerosis. *Autoimmun Rev* 2020; 19: 102494.

89. Mirone L, Barini A and Barini A. Androgen and prolactin (Prl) levels in systemic sclerosis (SSc): relationship to disease severity. *Ann N Y Acad Sci* 2006; 1069: 257–262.

90. Harbo HF, Gold R and Tintoré M. Sex and gender issues in multiple sclerosis. *Prog Brain Res* 2009; 175: 239–251.

91. Gold SM and Voskuhl RR. Estrogen and testosterone therapies in multiple sclerosis. *Prog Brain Res* 2010; 87: 237–248.

92. Reske D, Haupt WF and Petereit HF. Gender change and its impact on the course of multiple sclerosis. *Acta Neurol Scand* 2006; 113: 347–349.

93. Ysrraelit MC and Correale J. Impact of sex hormones on immune function and multiple sclerosis development. *Immunology* 2019; 156: 9–22.
94. Brubaker WD, Li S, Baker LC, et al. Increased risk of autoimmune disorders in infertile men: analysis of US claims data. *Andrology* 2017; 6: 94–98.

95. Pakpoor J, Goldacre R, Schmierer K, et al. Testicular hypofunction and multiple sclerosis risk: a record-linkage study. *Ann Neurol* 2014; 76: 625–628.

96. Bove R and Chitnis T. The role of gender and sex hormones in determining the onset and outcome of multiple sclerosis. *Mult Scler* 2014; 20: 520–526.

97. D’Amico E, Zanghi A, Burgio G, et al. Gonadal steroids and sperm quality in a cohort of relapse remitting multiple sclerosis: a case-control study. *Front Neurol* 2020; 11: 756.

98. Confavreux C, Hutchinson M, Hours MM, et al. Rate of pregnancy-related relapse in multiple sclerosis. *NEJM* 1998; 339: 285–291.

99. Sicotte NL, Liva SM, Klutch R, et al. Epstein-Barr virus and multiple sclerosis. *Front Immunol* 2019; 10: 35–64.

100. Abu-Raya B, Michalski C, Sadarangani M, et al. Maternal immunological adaptation during normal pregnancy. *Front Immunol* 2020; 11: 575197.

101. Leffler J, Trend S, Gorman S, et al. Sex-specific environmental impacts on initiation and progression of multiple sclerosis. *Front Neurol* 2022; 13: 835162.

102. Houen G, Trier NH and Frederiksen JL. Epstein-Barr virus and multiple sclerosis. *Front Immunol* 2020; 11: 587078.

103. Pakpoor J, Wotton CJ, Schmierer K, et al. Gender identity disorders and multiple sclerosis risk: a national record-linkage study. *Mult Scler* 2016; 22: 1759–1762.

104. Laffont S and Guéry JC. Deconstructing the sex bias in allergy and autoimmunity: from sex hormones and beyond. *Adv Immunol* 2019; 142: 35–64.

105. Castro C and Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. *J Allergy Clin Immunol* 2010; 125(2, Suppl. 2): S238–S247.

106. Sg R, Ingenito F, Ea M, et al. High prevalence of altered immunological biomarkers in a transgender population. *Autoimmun Infec Dis* 2020; 3: 1–5.

107. Shah S. Hormonal link to autoimmune allergies. *ISRN Allerg* 2012; 2012: 910437–910435.

108. Leffler J, Stumbles P and Strickland D. Immunological processes driving IgE sensitisation and disease development in males and females. *Int J Mol Sci* 2018; 19: 1554.

109. Jensen-Jarolim E. Gender effects in allergy – secondary publications and update. *World Allergy Organ J* 2017; 10: 47.

110. Lauzon-Joset JF, Mincham KT, Abad AP, et al. Oestrogen amplifies pre-existing atopy-associated Th2 bias in an experimental asthma model. *Clin Exp Allergy* 2019; 50: 391–400.

111. Arroyo AC, Sanchez DA, Camargo CA Jr, et al. Evaluation of allergic diseases in transgender and gender-diverse patients: a case study of asthma. *J Allergy Clin Immunol* 2022; 10: 352–354.

112. Strauss P, Cook A, Winter S, et al. Trans pathways: the mental health experiences and care pathways of trans young people. Summary of results. Telethon Kids Institute, Nedlands, WA, Australia, September 2017.

113. Strauss P, Lin A, Winter S, et al. Options and realities for trans and gender diverse young people receiving care in Australia’s mental health system: findings from trans pathways. *Aust N Z J Psychiatry* 2021; 55: 391–399.

114. Strauss P, Winter S, Waters Z, et al. Perspectives of trans and gender diverse young people accessing primary care and gender-affirming medical services: findings from trans pathways. *IJTH* 2021; 23: 295–307.

115. Rosenberg S, Callander D, Holt M, et al. Cisgenderism and transphobia in sexual health care and associations with testing for HIV and other sexually transmitted infections: findings from the Australian Trans & Gender Diverse Sexual Health Survey. *PLoS ONE* 2021; 16: 0253589.

116. Laffont S, Blanquart E and Guéry JC. Sex differences in asthma: a key role of androgen-signaling in group 2 innate lymphoid cells. *Front Exp Allergy* 2019; 50: 391–400.

117. Kim H-I, Lim H and Moon A. Sex differences in cancer: epidemiology, genetics and therapy. *Biomol Ther* 2018; 26: 335–342.

118. Folkerd EJ and Dowsett M. Influence of sex hormones on cancer progression. *J Clin Oncol* 2010; 28: 4038–4044.

119. Chengalvala MV, Pelletier JC and Kopf GS. GnRH agonists and antagonists in cancer therapy. *Curr Med Chem Anticancer Agents* 2003; 3: 399–410.

120. Mitchell C, Richards S, Harrison CJ, et al. Long-term follow-up of the United Kingdom
medical research council protocols for childhood acute lymphoblastic leukaemia, 1980–2001. Leukemia 2010; 24: 406–418.

121. Sterling J and Garcia MM. Cancer screening in the transgender population: a review of current guidelines, best practices, and a proposed care model. Transl Androl Urol 2020; 9: 2771–2785.

122. Braun H, Nash R, Tangprincha V, et al. Cancer in transgender people: evidence and methodological considerations. Epidemiol 2017; 39: 93–107.

123. Irelli A, Sirufo MM, D’Ugo C, et al. Sex and gender influences on cancer immunotherapy response. Biomedicines 2020; 8: 232.

124. Berghella AM, Secinaro E, Beato TD, et al. The role of gender-specific cytokine pathways as drug targets and gender-specific biomarkers in personalised cancer therapy. Curr Drug Targets 2016; 18: 485–495.

125. Velez MA, Burns TF and Stabile LP. The estrogen pathway as a modulator of response to immunotherapy. Immunotherapy 2019; 11: 1161–1176.

126. Wang S, Cowley LA and Liu X-S. Sex differences in cancer immunotherapy efficacy, biomarkers, and therapeutic strategy. Molecules 2019; 24: 3214.

127. Smolle MA, Calin HN, Pichler M, et al. Noncoding RNAs and immune checkpoints-clinical implications as cancer therapeutics. FEBS J 2017; 284: 1952–1966.

128. Dika E, Patrizi A, Lambertini M, et al. Estrogen receptors and melanoma: a review. Cells 2019; 8: 1463.

129. Beguerie JR, Xingzhong J, Valdez RP, and Tamoxifen vs. Non-tamoxifen treatment for advanced melanoma: a meta-analysis. Int J Dermatol 2010; 49: 1194–1202.

130. Reisner SL, Poteat T, Keatley J, et al. Global health burden and needs of transgender populations: a review. Lancet 2016; 388: 412–436.

131. Blok CJMd, Wiepjes CM, Nota NM, et al. Breast cancer risk in transgender people receiving hormone treatment: nationwide cohort study in the Netherlands. BMJ 2019; 365: 1652.

132. McFarlane T, Zajac JD and Cheung AS. Gender-affirming hormone therapy and the risk of sex hormone-dependent tumours in transgender individuals – a systematic review. Clin Endocrinol (Oxf) 2018; 89: 700–711.

133. Jacobsen H and Klein SL. Sex differences in immunity to viral infections. Front Immunol 2021; 12: 720952.

134. Mege JL, Bretelle F and Leone M. Sex and bacterial infectious diseases. New Microbes New Infect 2018; 26: S100–S103.

135. Kawai T and Akira S. TLR signaling. Cell Death Differ 2006; 13: 816–825.

136. Warrington R, Watson W, Kim HL, et al. An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 2011; 7: S1.

137. Petersone L, Edner NM, Ovcinnikovs V, et al. T cell/B cell collaboration and autoimmunity: an intimate relationship. Front Immunol 2018; 9: 1941.

138. Torcia MG, Nencioni L, Clemente AM, et al. Sex differences in the response to viral infections: TLR8 and TLR9 ligand stimulation induce higher IL10 production in males. PLoS ONE 2012; 7: e99853.

139. Bender AT, Tzvetkov E, Pereira A, et al. TLR7 and TLR8 differentially activate the IRF and NF-κB pathways in specific cell types to promote inflammation. Immunohorizons 2020; 4: 93–107.

140. Poteat T, Scheim A, Xavier J, et al. Global epidemiology of HIV infection and related syndemics affecting transgender people. J Acquir Immune Defic Syndr 2016; 72: 207–209.

141. Rechtien A and Altfeld M. Sexual dimorphism in HIV-1 infection. Semin Immunopathol 2019; 41: 195–202.

142. Fourati S, Flandre P, Calin R, et al. Factors associated with a low HIV reservoir in patients with prolonged suppressive antiretroviral therapy. J Antimicrob Chemother 2014; 69: 753–756.

143. Guéry JC. Sex differences in primary HIV infection: revisiting the role of TLR7-driven type 1 IFN production by plasmacytoid dendritic cells in women. Front Immunol 2021; 12: 729233.

144. Meier A, Chang JJ, Chan ES, et al. Sex differences in the toll-like receptor–mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med 2009; 15: 955–959.

145. Das B, Dobrowolski C, Luttge B, et al. Estrogen receptor-1 is a key regulator of HIV-1 latency that imparts gender-specific restrictions on the latent reservoir. PNAS 2018; 115: 795–804.

146. Vázquez-Martínez ER, García-Gómez E, Camacho-Arroyo I, et al. Sexual dimorphism in bacterial infections. Biol Sex Differ 2018; 9: 27.
147. Gay L, Melenotte C, Lakbar J, et al. Sexual dimorphism and gender in infectious diseases. *Front Immunol* 2021; 12: 698121.

148. Callander D, Cook T, Read P, et al. Sexually transmissible infections among transgender men and women attending Australian sexual health clinics. *Med J Aust* 2019; 211: 406–411.

149. Melendez RM and Pinto R. ‘It’s really a hard life’: love, gender and HIV risk among male-to-female transgender persons. *Cult Health Sex* 2007; 9: 233–245.

150. Karlberg J. Do men have a higher case fatality rate of severe acute respiratory syndrome than women do? *Am J Epidemiol* 2004; 159: 229–231.

151. Bignucolo A, Scarabel L, Mezzalira S, et al. Sex disparities in efficacy in COVID-19 vaccines: a systematic review and meta-analysis. *Vaccines* 2021; 9: 825.

152. Jin JM, Bai P, He W, et al. Gender differences in patients with COVID-19: focus on severity and mortality. *Front Public Health* 2020; 8: 152.

153. Onofrio L, Caraglia M, Facchini G, et al. Toll-like receptors and COVID-19: a two-faced story with an exciting ending. *Future Science OA* 2020; 6: FSO605.

154. Bunders MJ and Altfeld M. Implications of sex differences in immunity for SARS-CoV-2 pathogenesis and design of therapeutic interventions. *Immunity* 2020; 53: 487–495.

155. Levin AT, Hanage WP, Owusu-Boaitey N, et al. Assessing the age specificity of infection fatality rates for COVID-19: systematic review, meta-analysis, and public policy implications. *Eur J Epidemiol* 2020; 35: 1123–1138.

156. Van Der Made CI, Simons A, Schuurs-Hoeijmakers J, et al. Presence of genetic variants among young men with severe COVID-19. *JAMA* 2020; 324: 663–673.

157. Lopez E, Sang PC, Tian Y, et al. Dysregulated interferon response underlying severe COVID-19. *Viruses* 2020; 12: 1433.

158. Wang Z, Pan H and Jiang B. Type I IFN deficiency: an immunological characteristic of severe COVID-19 patients. *Signal Transduct Target Ther* 2020; 5: 198–199.

159. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 2020; 370: 6515.

160. Asano T, Boisson B, Onodi F, et al. X-linked recessive TLR7 deficiency in ~1% of men under 60 years old with life-threatening COVID-19. *Sci Immunol* 2021; 6: 4348.

161. Burgess CM, Batchelder AW, Sloan CA, et al. Impact of the COVID-19 pandemic on transgender and gender diverse health care. *Lancet Diabetes Endocrinol* 2021; 9: 729–731.

162. van der Miesen AIR, Raaijmakers D and van de Grift >TC. ‘You have to wait a little longer’: transgender (mental) health at risk as a consequence of deferring gender-affirming treatments during COVID-19. *Arch Sex Behav* 2020; 49: 1395–1399.

163. Klein SL, Marriott J and Fish EN. Sex-based differences in immune function and responses to vaccination. *RSTMH* 2014; 109: 9–15.

164. Fischinger S, Boudreau CM, Butler AL, et al. Sex differences in vaccine-induced humoral immunity. *Semin Immunopathol* 2019; 41: 239–249.

165. Dominguez A, Plans P, Costa J, et al. Seroprevalence of measles, rubella, and mumps antibodies in Catalonia, Spain: results of a cross-sectional study. *Eur J Clin Microbiol Infect Dis* 2006; 25: 310–317.

166. Poethko-Müller C and Mankertz A. Seroprevalence of measles-, mumps- and rubella-specific IgG antibodies in German children and adolescents and predictors for seronegativity. *PLoS ONE* 2012; 7: e42867.

167. Engler RJM, Nelson MR, Klotte MM, et al. Half- vs full-dose trivalent inactivated influenza vaccine. *JAMA Int Med* 2008; 168: 2405–2414.

168. Poturi T, Fink AL, Sylvia KE, et al. Age-associated changes in the impact of sex steroids on influenza vaccine responses in males and females. *Npj Vaccines* 2019; 4: 29.

169. DoH. COVID-19 vaccine rollout update – 9th May 2022, https://www.health.gov.au/resources/publications/covid-19-vaccine-rollout-update-14-april-2022 (2022, accessed 10 May 2022).

170. Clark TC, Lucassen MF, Bullen P, et al. The health and well-being of transgender high school students: results from the New Zealand Adolescent Health Survey (Youth’12). *J Adolesc Health* 2014; 55: 93–99.

171. Fisher CM, Waling A, Kerr L, et al. The 6th National Survey of Australian secondary students and sexual health. La Trobe University, Melbourne, VIC, Australia, June 2019.

172. Telfer MM, Tollit MA, Pace CC, et al. Australian standards of care and treatment guidelines for transgender and gender diverse children and adolescents. *Med J Aust* 2018; 209: 132–136.
173. Marshall WA and Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child* 1969; 44: 291–303.

174. Marshall WA and Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 1970; 45: 13–23.

175. Telfer M, Tollit M and Feldman D. Transformation of health-care and legal systems for the transgender population: the need for change in Australia. *J Paediatr Child Health* 2015; 51: 1051–1053.

176. Cundill P. Hormone therapy for trans and gender diverse patients in the general practice setting. *Aust J Gen Pract* 2020; 49: 385–390.

177. Strauss P, Cook A, Winter S, *et al.* Associations between negative life experiences and the mental health of trans and gender diverse young people in Australia: findings from trans pathways. *Psychol Med* 2020; 50: 808–817.

178. Coleman E, Bockting W, Botzer M, *et al.* Standards of care for the health of transsexual, transgender, and gender-nonconforming people, version 7. *Int J Transgend* 2012; 13: 165–232.

179. Hembree WC, Cohen-Kettenis PT, Gooren L, *et al.* Endocrine treatment of gender-dysphoric. *J Clin Endocrinol Metab* 2017; 102: 3869–3903.

180. Claahsen-van der Grinten H, Verhaak C, Steensma T, *et al.* Gender incongruence and gender dysphoria in childhood and adolescence – current insights in diagnostics, management, and follow-up. *Eur J Pediatr* 2021; 180: 1349–1357.

181. Allen LR, Watson LB, Egan AM, *et al.* Wellbeing and suicidality among transgender youth after gender-affirming hormones. *APA* 2019; 7: 302–311.

182. de Vries AL, McGuire JK, Steensma TD, *et al.* Young adult psychological outcome after puberty suppression and gender reassignment. *Pediatrics* 2014; 134: 696–704.

183. Peterson CM, Matthews A, Copps-Smith E, *et al.* Suicidality, self-harm, and body dissatisfaction in transgender adolescents and emerging adults with gender dysphoria. *Suicide Life Threat Behav* 2017; 47: 475–482.

184. Achille C, Taggart T, Eaton NR, *et al.* Longitudinal impact of gender-affirming endocrine intervention on the mental health and well-being of transgender youths: preliminary results. *Int J Pediatr Endocrinol* 2020; 2020: 8.

185. Jiang X, Ellison SJ, Alarid ET, *et al.* Interplay between the levels of estrogen and estrogen receptor controls the level of the granzyme inhibitor, proteinase inhibitor 9 and susceptibility to immune surveillance by natural killer cells. *Oncogene* 2007; 26: 4106–4114.

186. Bupp MRG and Jorgensen TN. Androgen-induced immunosuppression. *Front Immunol* 2018; 9: 794.