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Therapeutic use of selective synthetic ligands for retinoic acid receptors: a patent review

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Introduction: Differentiation therapy using all-*trans* retinoic acid (ATRA) revolutionised the treatment of acute promyelocytic leukaemia to the extent this leukaemia is one of the most curable with ATRA and anthracycline-based chemotherapy providing cure rates above 80% (reviewed in [1]). Isotretinoin is used to treat chronic acne. Here, we examine the information described in recent patents and the extent to which new findings are leading towards extending retinoid-based differentiation therapy to other cancers and the development of new therapies for other disorders.

Areas covered: A search has been undertaken of the literature and of worldwide patents, filed during 2014 to the present time, in regard to synthetic agonists and antagonists of retinoic acid receptors and novel compositions for the delivery of these agents.

Expert opinion: New potential therapeutic applications have been described, including lung, breast and head and neck cancers, T cell lymphoma and neurodegenerative, metabolic, ophthalmic, muscle and inflammatory disorders. Recent patents have described the means to maximise retinoid activity. Two decades of efforts to extend retinoid-based therapies have been disappointing and new synthetic retinoids, target diseases and modes of delivery may well resolve this long standing issue.

Highlights

● There are highly selective agonists for all three RAR subtypes and antagonists except for RARβ.

● The tissue distribution of RAR subtypes is variable and functional redundancy is not the case.

● New patents describe the prospects of extending ATRA-driven differentiation therapy of acute promyelocytic leukaemia to other cancers.

● New patents have examined the prospects of broadening the therapeutic uses of retinoids to neurodegenerative, metabolic, ophthalmic, muscle and inflammatory disorders.

● New drug composition and delivery methods are important to improving the efficacy of retinoids.

1. Introduction

Natural retinoids are the ligands to different subtypes of retinoic acid receptors (RARs) and retinoid X receptors (RXRs). They are metabolically activated derivatives of vitamin A, which is delivered to the body by dietary sources. Active metabolites include the all-*trans*-, 9-*cis*- and 13-*cis*-retinoic acids (ATRA, 9cRA and 13cRA, respectively). The predominant natural retinoid is ATRA, which is present in most tissues and in blood serum. The one less abundant but detectable in almost all tissues is 13cRA. 9cRA is more difficult to detect, and the only organ in which currently available methods detect this metabolite is pancreas [2]. Retinoic acids play crucial roles in embryonic development and as to the behavior of adult tissues. In both these circumstances, retinoids are key modulators of cell growth, differentiation and apoptosis through their signaling pathways [3]. The different subtypes of RAR, their tissue distribution and the availability of novel synthetic analogues of retinoids that selectively agonize and antagonize a RAR subtype underlie the precise therapeutic use of retinoids. As outlined below, the tissue expression of subtypes is variable and functional redundancy is not the case in regard to the major subtypes.

2. Actions of retinoids *via* nuclear receptors

The biological activities of retinoids are mediated in target cells through two different classes of nuclear receptors: RARs and RXRs which are encoded by separate sets of genes [4]. There are three subtypes of RARs and of RXRs which are designated as α, β and γ. Several isoforms are generated by alternative splicing or alternative transcriptional start sites. In 2006, the International Union of Pharmacology recommended official names for these receptors and their subtypes. The agreed names for RARα, RARβ and RARγ are NR1B1,

NR1B2 and NR1B3, respectively, and for RXRα, RXRβ and RXRγ are NR2B1, NR2B2 and NR2B3, respectively. The trivial, and more frequently used, names have been used throughout this manuscript. RARs and RXRs differ as to their ligand specificity. ATRA binds to and activates RARs and has a similar affinity for all three subtypes. 9cRA binds to and activates all three RARs and RXRs with different affinities [5]. The dual activation of RARs and RXRs by 9cRA is related to its flexible structure which allows conformational adaptation to the different binding pockets [6].

Like most members of the nuclear receptor superfamily, RARs are ligand activated transcription factors. They form heterodimers with RXRs and function as ligand-inducible transcription factors by binding to DNA sequences called retinoic acid response elements (RARE) within the promoter region of a target gene [5]. Activity of the heterodimer is controlled by RAR once ligand is bound; however RXR ligand can increase the transcriptional efficiency of the heterodimer [7]. The various isoforms of RAR and RXR create numerous receptor combinations. Moreover, the ability of RXRs to form heterodimers with numerous other nuclear receptors and to modulate a wide range of specific genes adds another level of complexity [8]. RARs can either repress or activate transcription [9,10]. Some of them act as repressors of transcription in the absence of the ligand, and upon binding of ligand the transcriptional repressors are released from the receptor complex and the interface for co-activators becomes accessible. Histone acetylase and RNA polymerase belong to the co-activation (CoA) complex, and, therefore, the chromatin structure becomes loose and the transcription of the target gene can start [11].

Recent observations suggest that, in addition to RARs, the retinoic acid signaling pathway involves the peroxisome proliferator–activated receptor β/δ (PPAR β/δ) [12]. PPAR β/δ is a

subtype of the PPARss which are ligand-activated nuclear receptors that are stimulated by small lipophilic ligands [13]. PPAR β/δ is expressed ubiquitously, with high expression observed in the brain, adipose tissue, skeletal muscle and skin [14]. Similar to RARs, activated PPAR β/δ forms a heterodimer with RXR and binds to specific PPAR response elements (PPREs) in the promoter regions of target genes [15]. Therefore, RARs and PPAR β/δ can activate different set of target genes and exert opposing activities. It has been shown that RARs trigger differentiation, cell cycle arrest and apoptosis [16], while PPAR β/δ promotes cell survival and proliferation [17,18]. A dual action of retinoic acid is related to different partitioning inside the cell between the two receptors. This process is regulated by the intracellular lipid binding proteins: cellular retinoic acid binding protein II (CRABP-II), and fatty acid binding protein 5 (FABP5) which deliver retinoic acid to RARs and PPAR β/δ, respectively Thus, the cellular retinoic acid response depends on the CRABP-II/FABP5 ratio [19].

3. Tissue distribution of receptors

There are important differences as to the tissue distributions of RARs and RXRs. In humans, RXRβ is ubiquitously expressed; RXRα is mainly expressed in the liver, lung, muscle, kidney, epidermis, and intestine and it is the major subtype in skin; and RXRγ is found in the brain, cardiac and skeletal muscle. As for the RARs, RARα has a widespread expression pattern. In contrast, RARβ expression is prevalent in neural tissues and hardly detectable in skin; and RARγ is expressed predominantly in the skin [20,21]. The distribution of RAR subtypes and their isoforms has been studied in different species using a variety of techniques. An overview of the distribution of RARs in different organs is presented in Table 1 and details of these studies are provided in the following sections. Besides physiological roles in normal tissues, retinoid receptors also play a role in the development of diseases

including cancers. Such is often due to mutations, chromosomal translocations, change to the level of expression, aberrant post-translational modifications, and epigenetic changes. These events result in altered function leading to disruption of homeostasis [22]. The abnormalities in retinoid signaling have been related mostly to dysregulation of RARα and RARβ [23].

4. Subtypes and isoforms of retinoic acid receptors and their tissue distribution

4.1. Retinoic acid receptor α

The *RARA* gene is located on chromosome 17 and is composed of 10 exons and two promoters that give rise to the two isoforms RARα1 and RARα2 [24]. RARα1 has a broad tissue distribution, including the liver, spleen, kidney, prostate, spinal cord, cerebral cortex, uterus, ovary, testis and breast, and is considered as a canonical isoform [25]. In contrast, RARα2 is expressed in a limited number of tissues, for example, the intestine, lung and liver, and appears in the absence of ligand to be a more potent inhibitor of cell differentiation than RARα1. Its role in maintaining cells in an undifferentiated stem cell state [26] is seen in multiple myeloma whereby RARα2 expression is linked to primary multiple myeloma and drug resistance in this disease [27].

Cells of the hematopoietic system express mostly the RARα and RARγ subtypes and each has a specific modulatory role [17]. Although, disruption of any one of these two genes does not alter hematopoiesis, RARα deficient mice demonstrated an impaired response to retinoids which leads to the accumulation of more immature granulocytes in their bone marrow after vitamin A treatment. Thus, RARα can modulate granulopoiesis in response to retinoids [28]. Moreover, the double mutant mice RARα$^{-/-}$RARγ$^{-/-}$ die *in utero* and, therefore, it is hard to conclude whether RARs are needed for adult bone marrow hematopoiesis. However,

disruption of both genes affects granulocyte differentiation potential. The double mutant $RAR\alpha1^{-/-}RAR\gamma^{-/-}$ cells derived from foetal liver were found to be blocked at the myelocyte/metamyelocyte stage of myelopoiesis [29]. On the other hand, $RAR\alpha^{-/-}RAR\gamma^{-/-}$ double mutant cells differentiate faster in response to G-CSF and SCF. These inconclusive results may be related to effects on different $RAR\alpha$ isoforms and implicate distinctions between the functions of $RAR\alpha1$ and $RAR\alpha2$. Oren and co-workers have suggested that $RAR\alpha2$ may more effectively inhibit differentiation, and this becomes the predominant phenotype in $RAR\alpha1^{-/-}$ cells [30]. Nevertheless, Zhu and co-workers demonstrated for murine progenitor cells that $RAR\alpha$ upregulation is required for optimal differentiation of primitive myeloid cells to granulocytes [31] and activity of $RAR\alpha$ is altered, by chromosomal translocations, in all cases of acute promyelocytic leukaemia (APL). A characteristic of this type of leukemia is the leukaemic cells are blocked at the promyelocyte stage of granulocyte differentiation [32]. The chromosomal translocation that fuses the PML gene to the $RAR\alpha$ gene gives rise to the protein PML-$RAR\alpha$ which acts as a constitutive transcriptional repressor [31]. However, a pharmacological dose of ATRA is able to dissociate co-repressors from the PML-$RAR\alpha$ bound to DNA to allow activation of transcription and, therefore, the differentiation of APL cells into mature granulocytes [33]. Resistance of leukemia cells to the differentiating effect of ATRA has also been correlated with aberrant or deficient phosphorylation of $RAR\alpha$ [34]. Appropriate phosphorylation of $RAR\alpha$ is important to retinoid signaling and activated RARs provoke signaling via non-genomic pathways by activating p38MAPK and its downstream target mitogen and stress-activated kinase 1(MSK1). These events are required for cell differentiation. Additional disruption of $RAR\alpha$ has been observed in cells from acute myeloid leukemia (AML) patients whereby histones that associate with the *RARA2* promoter show a decrease in their overall acetylation and a

decreased level of dimethylation. These findings reveal that epigenetic changes to the landscape of *RARA* gene are involved in AML pathogenesis [33].

As to the involvement of RARα in carcinomas, some estrogen receptor (ER)-negative breast cancer cells are resistant to retinoic acid and these cells have a reduced level of expression of RARα; overexpression of RARα can restore ATRA-driven growth inhibition. RARα can also participate in estrogen-mediated proliferation as RARα has been shown to be induced by estrogens and shares a subset of binding regions with the estrogen receptor and, therefore, can be part of the estrogen receptor transcriptional complex [22]. As to ovarian cancer, the level of expression of RARα has been correlated with ATRA-driven growth inhibition of ovarian cancer cell lines, but not for primary tumors [35]. RARα can act also as an oncogene. This occurs in hepatocellular carcinoma whereby in the absence of the corepressor transcriptional intermediary factor 1α (TIF1α) oncogenesis correlates with the deregulation of retinoic acid signaling [36].

Recently is has been shown that retinoic acid is necessary for the function of the brain and new discoveries point to a crucial role in synaptic plasticity, learning and memory behaviors. RARα, RARβ and RARγ have been detected in the adult brain. RARα has a widespread distribution with high levels in the hippocampus, cerebellum and cortex. In contrast, RARβ has a more restricted distribution that includes high levels in the striatum and the spinal cord, and RARγ is found at a low level in the hippocampus [37]. Recent findings suggest that age-related neuron loss, impairment of memory and cognition and even some neurodegenerative disorders are related to the disruption of retinoic acid signaling [37].

4.2. Retinoic acid receptor β

The *RARB* gene is located on chromosome 3 [38]. It is composed of 13 exons and 4 promoter regions which give rise to five distinct isoforms [39]. Expression of RARβ is rapidly induced by ATRA whereas expression of RARα and RARγ is sustained within cells at relative constant levels. In keeping with this observation, a RARE has been identified in the promoter region of RARβ2 [40] and RARα/RXR dimers bind to resulting in the expression of RARβ2 [41]. Differences in the usage of *RARB1* and *RARB2* promoters and alternative splicing give rise to the major RARβ isoforms β1, β2, and β4, and additional isoforms, such as RARβ5 and RARβ1', have been identified [42]. RARβ plays a key role in multiple diseases [5] and the various RARβ isoforms have different affinities for ATRA (at least with regard to RARβ2 and RARβ4) and different biological functions. For example, the RARβ2 protein is a tumor suppressor whereas RARβ4 has oncogenic properties [42].

Unlike RARα, the tissue distribution of RARβ is more restricted and it is prevalent in epithelial tissues [40] and neural tissue and hardly detectable in skin [23]. RARβ1 is considered to be a foetal isoform and plays a crucial role during development [39]. Whereas RARβ1 is largely undetectable in adult tissues aberrant expression has been observed in several lung-cancer derived cell lines [38]. The recently identified RARβ1' isoform, which arises from an alternative splicing of RARβ1, is expressed in normal lung tissue and bronchial epithelial cells and the level of expression is suppressed in human lung cancers that are resistant to ATRA. Transfection of ATRA-resistant lung cancer cell lines and bronchial epithelial cells with RARβ1' restored ATRA-sensitivity [43]. These data suggest that RARβ1' plays a critical role in mediating the biological effects of retinoids in relation to carcinogenesis and may function as a tumor suppressor which is distinct from the observed function of RARβ2 [38,44].

The RARβ2 isoform is the major ATRA-inducible isoform of RAR [42] and it is considered as a canonical isoform of RARβ [43]. Loss of RARβ2 activity, especially relating to gene hyper-methylation, has been observed in many different types of cancers, including head, neck [45] colon [46] non-small lung cancer [47], cervical cancer [44], breast [48] and prostate cancer [49]. Other studies have shown that transcriptional deregulation can silence RARβ2 expression through decreased levels of co-activators, the presence of co-repressors or epigenetic mechanisms such as histone deacetylation. Expression of RARβ2 also depends on the cellular level of retinoids as to the ATRA-inducible nature of this RAR [42]. Experiments *in vitro* as well as *in vivo* support the hypothesis that the ATRA growth inhibitory action is lost with impaired RARβ2 expression. Berard and co-workers observed a higher incidence of pulmonary tumors in a truncated RARβ2 mouse model. The endogenous RARβ2 message level was found to be reduced in transgenic lung tissue and further reduced in the tumours [50]. Moreover, disruption of RARβ2 in the teratocarcinoma cell line F9 impaired retinoic acid–mediated growth inhibition and differentiation [51]. On the other hand, endogenous reactivation of the RARβ2 gene, by chromatin remodeling drugs, in breast cancer cell lines and xenograft tumors restored retinoic acid–dependent growth inhibition [52]. All of the above strongly suggest that RARβ2 acts as tumor suppressor and loss of its expression may be an early and common event during malignant progression [41].

The RARβ4 isoform is a splice variant of RARβ2 and has a distinct functional attribute [53]. It lacks a DNA binding domain, and is unable to activate the transcription of target genes. Therefore, it has a dominant negative role [54]. ATRA-resistance of breast cancer cells is associated with down-regulation of RARβ2 [55] and over-expression of RARβ4. Findings from studies of breast cancer cells support the viewpoint that RARβ2 is a potent inhibitor of

cell proliferation and that RARβ4 interferes with RARβ2-mediated growth suppression upon treatment of cells with ATRA [56].

The RARβ5 isoform is similar to RARβ4 and is a splice variant of RARβ2 that lacks the domains A and B and part of domain C. RARβ5 is able to form a functional hetero-complex with RXRs, but, as is the case for RARβ4, RARβ5 cannot bind to DNA and activate transcription of target genes [57]. RARβ5 was initially found in epithelial cells but it is also expressed in breast epithelial cells and benign, premalignant and tumor cell lines. Moreover, it is preferentially expressed in estrogen-negative breast cancer cells that are resistant to the anti-proliferative action of retinoids [57].

4.3. Retinoic acid receptor γ

The *RARG* gene is located on chromosome 12. The major portion of the RARγ protein, including the DNA and ligand binding domains, is encoded by seven exons that are identical for the RARγ1 and RARγ2 isoforms. These isoforms differ only in their N-terminal regions, the N-terminal region of RARγ2 shows high homology with that of RARβ and transcription of RARγ2 is regulated by its own promoter [58]. Lehmann and colleagues have shown that RARγ2, like RARα2 and RARβ2, is activated by retinoids and RARγ1 is not able to drive ligand-dependent transcription. The poor transactivation function of RARγ and ability to compete for DNA binding suggest that a major function of RARγ1 is to suppress gene activation by other RARs [59].

RARγ1 is predominantly expressed in skin where RARγ2 expression is low [60]. Both RARα and RAR*γ* show differential expression throughout the epidermal layers whereby expression of RARα and RAR*γ* is much higher in the spinous and granular layers in comparison to the

basal layer of epidermal cells [61]. In the case of keratinocytes, RARγ is considered to be a tumor suppressor gene, as RARγ was found to be absent from oral keratinocytes obtained from head and neck cancers. In addition, the incidences of premature skin aging and skin cancer, induced by ultra-violet radiation, have been correlated to a dramatic decrease in the level of RARγ, due to proteasomal degradation of the receptor [22]. RARγ can also act as an oncogene. In hepatocellular carcinoma, overexpression of RARγ correlates with an increased cell survival through its altered subcellular localization. In this case, there is strong cytoplasmic localization of RARγ which interacts with the phosphatidylinositol 3-kinase (PI3K) regulatory subunit p85α leading to the activation of the PI3K/Akt pathway, which is one of the major survival pathways in cancer cells [62].

Though RARγ has been found to be mainly expressed in skin, RARγ is viewed as a critical regulator of the balance between haematopoietic stem cells (HSC) being able to maintain their stem cell status and these cells embarking on differentiation to produce mature blood cells [22]. The γ-knockout mouse has a reduced number of HSCs and loss of RARγ also abrogated the capacity of ATRA to potentiate the maintenance of HSC in culture [63]. Agonizing RARγ appears to promote self-renewal and/or proliferation of HSCs and, as such, opposes the ligand-driven action of RARα to drive cells to differentiate. HSCs are still present in the knockout mouse and, like RARα, the role of RARγ is modulatory. Further evidence to support the notion that RARγ is important to allowing cells to maintain their pluripotency comes from studies of the generation of induced pluripotent stem cells (iPSC) from somatic cells. The efficiency by which these cells can be generated can be increased by the addition of RARγ to the Yamanaka cocktail of transcription factors used to generate iPSC [64].

5. Selective retinoic acid receptor ligands

Selectivity of ligand binding is conferred by the structure of the receptor's ligand binding domain (LBD). In the case of all RAR subtypes, LBD sequences are conserved as they differ only by three amino-acid residues. These differences are responsible for the different affinities of natural retinoids to RAR subtypes, and they allow the prospect of synthetic retinoids that are selective towards a particular subtype [65]. In the case of nuclear receptors, binding of the natural ligand to LBD induces conformational change which facilitates the binding of CoAs [66]. LBDs are composed of 12 α-helices and two short β-strands. After binding of the activating ligand the most mobile of all helices, helix H12 seals the ligand-binding cavity and creates the interface for binding of the transcriptional CoAs [65]. Some nuclear receptors, for example RARα, act as transcriptional repressors in their un-liganded (apo) form. This is due to the short C-terminal part of H10, which adopts a β-strand conformation and creates a surface for interaction with transcriptional co-repressors (CoR) [67]. Upon binding of agonist, this region adopts a helical structure (H11), and promotes displacement of H12 into an active position. However, some synthetic ligands bind to RARs with high affinity but fail to stabilize helix H12 in an active position. These ligands are classified as partial agonists or antagonists, depending on their ability to prevent CoA recruitment. Antagonists not only destabilize the position of helix H12, but also prevent natural ligands from binding to LBD [66]. Some RAR antagonists, which are particularly efficient in stabilizing the β-strand conformation and favour CoRs binding, are defined as inverse agonists [67,68]. The possibility of fine-tuning the responses of particular RAR subtype and that isoforms are differentially expressed in tissues allows the therapeutic effects of synthetic retinoids to be directed against the required organs, and, accordingly, limit unwanted side-effects.

6. Therapeutic Applications of Retinoids

Longstanding interests as to development of the retinoid-based therapies have focussed attention to some extent on dysregulation of RAR-mediated signalling in leukaemia and other cancers and that skin cells express RARs and the prospect of treating a variety of skin disorders. In these two areas, patented developments within the retinoid field have delivered significant clinical benefits. Of particular importance is the standard use of ATRA and isotretinoin to treat APL and chronic acne, respectively. Recent patented findings bring about improvements to these existing applications and anticipate a broadening of the potential therapeutic use of retinoids as outlined below. The patents examined in this review are listed in Table 2 and structures of compounds are shown in Figure 1.

6.1. Dermatological Conditions

Retinoids are efficacious as to the treatment of dermatological conditions. Isotretinoin (13*cis*RA), an orally administered drug, is successfully used to treat severe forms of acne such as acne vulgaris, for which antibiotic and other retinoid treatments have failed. However, 13*cis*RA causes dry skin and is teratogenic. This retinoid can be given to women of child bearing age but contraception has also to be given 1 month before treatment, during the treatment and for one month after. Two contraception methods should ideally be introduced. Depression has also been associated to a low level with taking 13cRA [69]. However, this remains controversial [70] and recent studies have contradicted this association [71]. Taken together the side effects and patient compliance are restricting factors as to use. For some acne sufferers, less toxic retinoids are efficacious. Tazarotene (AGN190168) is a cream composition of a RARβγ agonist that was developed by Allergan and alleviates acne in many patients. Retinoids are also a common ingredient to many cosmetic creams. Further applications of retinoids in dermatology include the use of acitretin, an oral retinoid, in the treatment of ichthyosis, lichen planus and psoriasis [72]. Acitretin is teratogenic and,

therefore, contraception has to be given to women of child bearing age 1 month before treatment, during the treatment and for 2 years after. Additionally, the RXR agonist bexarotene (LGD-1069) was licenced by the FDA in 2000 for use as an oral and topical treatment for cutaneous T-cell lymphomas and its associated lesions [73]. This compound can also bind to and activate RARs. Non-hormonal contraception is required in the case of LGD-1069 because medicament-activation of metabolic enzymes decreases the efficacy of hormonal contraception. A concern is that cases of hypothyroidism have been reported in patients given LGD-1069 [74,75]. The various retinoid-associated side effects lead to careful consideration of patient suitability, compound selection and regular monitoring of patients receiving treatment.

There has been little recent advancement with regard to new synthetic retinoids that are RAR subtype -selective for dermatological treatments. However, there have been advances regarding retinoid boosters. Granger and colleagues had demonstrated the ability of boosters to enhance the conversion of retinol and retinyl esters to retinoic acid in drug compositions, to enhance drug action [76-85]. More recently, Granger has described compositions of retinoid boosters that optimise the synergistic dermatological effects and has provided the respective concentrations that are required to maximise retinoid activity [86]. Interestingly, one new combination of tazarotene and dapsone (5% w/w), a neutrophil chemotaxis suppressant, resulted in a 13% greater reduction in acne associated comedonal lesions as compared to tazarotene alone. Additionally, combinations of tazarotene with either clindamycin (1% w/w) or benzyl peroxide (5% w/w) provided a 20% reduction in comedonal lesions in individuals.

6.2. Cancer

The most clinically significant application of retinoids to date is undoubtedly the use of ATRA to treat APL by overcoming the block to cell differentiation in this disease. What was once an untreatable illness is no longer so [87]; ATRA is the first in-line treatment for APL and often complemented with maintenance chemotherapy in the event of relapse or following refractoriness to ATRA. Considering this success and that RARs play an important role in regulating cell differentiation, proliferation and survival [88,89], there are ongoing efforts to extend ATRA differentiation therapy to other cancers. A limiting factor to use of ATRA to treat other cancers is an appreciable level of toxicity which can lead to fatal retinoic acid syndrome [90]. Moreover, extending the mechanism of ATRA's action in APL to other cancers is uncertain as to success in APL relates to the specific presence of the PML-RARα fusion protein that blocks cell differentiation (see above and [32]). As to both APL and other cancers there is a need to develop retinoids that avoid the toxicities associated with ATRA and RAR subtype -selective analogues might provide the answer.

Recent findings indicate the use of retinoids in other leukaemias. Churchman and colleagues reported recently that alterations to the IKZF1 gene are linked to a stem cell-like phenotype and increased cell adhesiveness in BCR-ABL1-associated acute lymphoblastic leukaemia pre-B cells [91]. A consequence of these alterations was reduced responsiveness to tyrosine kinase inhibitor (TKI) therapy. A reversal of the stem cell phenotype could be achieved by disrupting cell clustering. Four hundred and eighty-three compounds were tested and revealed that ATRA, 9cRA, 13cRA and LGD-1069 are potent inhibitors of cellular aggregation, as observed by an abrogation of the formation of spheres in Arf$^{-/-}$ BCR-ABL1 IK6-expressing pre-B cells. The retinoids and LGD-1069 also selectively induced expression of IKZF1 target genes and reduced colony forming potential, with LGD-109 significantly increasing cell responsiveness to TKI therapy.

There is the potential use of retinoids that are RAR subtype-selective to broaden retinoid treatment of cancer as evidenced by abnormal RAR signalling contributing to the pathogenesis of various carcinomas. Yan and colleagues have identified elevated levels of expression of RARγ in hepatocellular carcinoma cell lines and primary tumours and the growth stimulatory effect of such was alleviated by treating these cells with ATRA [62]. Also, RARγ has been shown to mediate ATRA-induced growth arrest and apoptosis of neoplastic mouse papilloma cell lines. Already, RAR subtype-selective retinoids have demonstrated their suitability for the treatment of malignancies other than APL and some are non-toxic [92,93]. One group has examined the possible use of retinoids to treat head and neck cancer (HNC). HNC comprises of cancers of the lips, larynx, pharynx, oral cavity, nasal cavity and treatment largely relies on surgery with the serious associated risks of perturbations to speech and the ability to swallow [94]. Previously, the RXR agonist LGD-1069 has shown potential efficacy in treating human T-cell lymphoma and lung cancer [92,93,95], and RARγ agonists in supressing tumour growth in mouse epidermal keratinocytes [96]. Gudas and colleagues have tested these agents individually and in combination in C57BL/6 mice bearing nitroquinoline-1-oxide (4-NQO)-induced oral cancers and significant benefit was observed [97,98]. Notably, gene expression changes induced by 4-NQO were prevented by LGD-1069 and the RARγ agonist. Additionally, the agents did not increase triglycerides and supressed reactive oxygen species production; Gudas suggested that these effects aid inhibition of tongue carcinogenesis. As to tumour migration, a combination of the above agents led to down-regulation of mRNA levels for a number of metallomatrix proteases (MMPs) as well as the protein levels of MMP9, which point to a potential strategy for preventing metastasis. The inventors additionally used a tongue carcinogenesis model to show that LGD-1069 and CD-1530 inhibited HIF1α signalling, a

pathway that regulates carcinogenesis, tumour development and migration [99] and overexpression of HIF1α, which is known to relate to poor prognosis in HNCs [100]. Further indicators of effectiveness included a reduction in the level of β-catenin, which is normally increased in oral squamous cell carcinoma [101,102], fewer cancer stem cells within the oral cavity and reduced tongue tumour development in general.

In vitro results have indicated the potential efficacy of retinoids in treating breast cancer, but this has still to translate to an effective treatment for patients [103]. In contrast, histone deacetylase inhibitors (HDACi) have demonstrated efficacy in pre-clinical studies of breast cancer [104,105]. Additionally, HDACi have been found to synergise with retinoids to suppress the growth of breast cancer cells [106]. Miller and colleagues have created a hybrid molecule that is a derivative of TTNN (6-(5, 6, 7, 8-tetrahydro-5, 5, 8, 8-tetramethyl-2-naphthalenyl)-2-naphthalenecarboxylic acid, see Figure 1), which agonises both RARβ and RARγ and inhibits histone deacetylase [107]. Hybrid 3 has been shown to have anti-proliferative effects *in vitro* against three breast cancer cell lines; MCF-7, SkBr3 and MDA-MB-231. Interestingly, the molecule was also shown to be active against the BT-20 breast cancer line which is double-negative for expression of ER and HER2 and insensitive to ATRA. Minimal effects were observed against non-tumour and normal mammary epithelial cells creating hope of a potential new treatment for breast cancer which appears not to affect normal breast cells.

6.3. Neurodegenerative Disorders
The pathology of Alzheimer's disease (AD) has been attributed to the deposition of amyloid plaque in neurological tissues [108]. The plaques comprise of amyloid β (Aβ) peptides [109,110], most of which are either Aβ 1-40 or Aβ 1-42 [111]. Critical to the creation of Aβ

peptide is the α-, β- and γ-secretase family of cell surface proteolytic enzymes. Sequential processing of amyloid precursor protein (APP) by β- and γ-secretases generates the pathologic Aβ peptides, whereas APP interaction with α-secretase, prior to β-secretase, yields a non-pathologic peptide. A disintegrin and metalloproteinase 10 (ADAM10) is the predominant proteolytic enzyme having α-secretase activity [112]. Increasing ADAM10 levels is, therefore, of interest as a therapeutic approach to AD. Retinol and its derivatives are known regulators of Aβ peptide formation and stimulate an increase in α-secretase activity [113]. A recent innovative combination of a byrostatin and ATRA, incorporated into a microsphere composition, has yielded an increase in α-secretase production in SH-SY5Y neuroblastoma cells as compared to bryostatin-1 treatment alone [114]. This novel finding points to the potential use of RAR-selective retinoids in combination with a bryostatin-1 for the treatment AD to mediate elevation of ADAM10.

There is evidence of a defect in retinoid transport in the brains of Alzheimer's patients [115] and the RAR agonist Am80 has been shown to reduce the level of Aβ in the brain of 5 month old APP23 mice [116]. Kawara and co-workers have pursued the avenue followed by Gudas and co-workers in the case of HNC [97] and examined the benefit to Alzheimer's patients of an RAR agonist used in combination with an RXR agonist [117]. Previously, LGD-1069 was found to enhance apolipoprotein E-dependent Aβ clearance alone [116] and the RAR agonist Am80 in addition to the RXR agonist HX630 reduced the level of Aβ significantly and strikingly reversed the defects in memory and spatial learning. As to the mechanism of repair, likely effectors include increased expression of the Aβ degrading enzymes, insulin degrading enzyme (IDE) and nuclear export protein (NEP).

Cell based therapies have been proposed as a non-drug method of promoting repair within the brain and neural tissues of individuals affected by neurodegenerative disorders [118].

However, there is limited availability of healthy brain tissue for obtaining neural stem cells and embryonic and iPSC are seen as an alternative source. Agonizing RARγ is known to enhance the frequency of reprogramming of somatic cells to iPSC [64]. The use of iPSC to repair neuronal tissue is dependent on the means to drive iPSC towards neuronal development. The embodiments of a new patent describe the use of ATRA to induce human trophoblast stem (hTS) cells to differentiate into neural cells, creating the potential for *in vitro* testing of drug efficacy and safety and the development of cellular therapies for neurodegenerative conditions [119]. Post-treatment of hTS cells with 10μM ATRA, dopaminergic neurons, glutamine neurons, serotonergic neurons or GABA (γ- aminobutyric acid) neurons expressing the neural markers neurofilament, nestin and glial fibrillary acidic protein were obtained. An embryonic or iPSC based therapy has not been licenced for use to date, but this development implies a contribution that retinoids might provide to neural cell therapies and more broadly to the field of regenerative medicine.

6.4. Metabolic Disorders

The consequences of a high fat diet and/or vitamin A deficiency can be severe. Heart, kidney, liver, testes and pancreatic disease, as well as cancer, stroke, and diabetes are some of the common complications. The results contained in a recent patent position selective RARβ agonists as a potential medicament for such ailments [120]. Following administration of retinoids capable of agonizing RARβ, such as; AC261066, AC55649, LE-135, AGN190168, CD-271, CD-666, 9cRA, BMS641 and AGN191183 significant improvements to indicators of the aforementioned conditions were observed. Notably, in murine models of diabetes and other pancreatic diseases, insulin and glucagon sensitivities were either maintained or improved, and β-cell degeneration was supressed. In the case of liver disease, decreases in alpha smooth muscle actin and hepatic reactive oxygen species as well as suppression of

hepatic stellate cell activation were observed, highlighting an ability to modulate the inflammatory response. The latter was further indicated by a reduction in inflammatory markers, such as monocyte chemotractant protein 1 and TNFα. Additionally, RARβ agonist treatment was found to increase lethicin:retinol acyltransferase (LRAT) and decrease sterol regulatory element binding protein 1c levels in the liver. Of particular note, the highly specific RARβ agonist AC261066 led to reduced liver steatosis, indicating a preventative role in fat accumulation. The inventors also found that agonism of RARβ could restore vitamin A signalling in vitamin A deficient organs such as the liver. Another interesting finding was triglyceride levels were not found not to be elevated suggesting a safe way of targeting high fat diet associated conditions. One of the long term considered risks with the therapeutic use of retinoids has been their tendency to elevate serum triglyceride levels [121-123], leading to a risk of heart disease. While this patent describes LE-135 as a RARβ agonist it should be noted that a previous report has described the compound as having RARβ antagonist activity [124].

6.5. Graft versus host disease

Graft versus host disease (GVHD) is a complication of allogeneic haematopoietic stem cell transplantation in which donor T cells within the graft recognise host antigens as foreign resulting in T cell activation. This condition can be fatal, with the three year survival rates for grade 1 GVHD being 58% and 30% above grade IV [125]. ATRA regulates T cell differentiation in a bimodal manner. Early on post-antigen stimulation of T cells, ATRA signalling supresses CD4+ve T cell differentiation towards T regulatory cells and provokes differentiation towards T helper 17 (Th17) and, thus, an inflammatory response. In contrast, ATRA signalling at a late stage during T cell responses supresses the Th17 pro-inflammatory response. Given that vitamin A metabolism is upregulated during GVHD, a heightened level of ATRA-driven RARα signalling exacerbates GVHD. That RARα signalling is required for

acute GVHD [126] means that antagonism of RARα is an interesting approach to ameliorating the side effects of or preventing GVHD. The embodiments of a new patent describe the use of the RAR selective antagonists, specified in US patents 5952345 and 5958954, to treat GVHD [127]. Inhibiting ATRA signalling in donor T cells attenuated GVHD lethality and importantly allowed the graft versus leukaemia effect to remain. Attenuation of ATRA signalling in GVHD patients or in patients immediately prior to transplant may, therefore, prove a useful means to manage and/or prevent GVHD. Indicated in the patent is that either a RARα or RARγ antagonist may be used, alone or in combination with an RXR agonist, to modulate the responsiveness of T cell donor cells. Given the bimodal action of selective retinoids in promoting and supressing T cell inflammatory responses, a great deal of care will be required to ensure safe manipulation of the T cell response.

6.6. Ophthalmic Disorders

Keratoconjuntival disorders are inflammatory conditions that affect the cornea and conjunctiva. Defects to either can negatively impact on the other and the damage can be serious. Keratoconjunctival disorders follow long delays as to the recovery from ophthalmic conditions such as dry eye (sicca), keratitis, infection or corneal ulcers due to a secondary injury. Degradation of the stromal element of the corneal parenchymal tissue and particularly of collagen type I is a key element of the pathogenesis. The delay in repair and degradation to collagen results in scar formation which can obstruct vision. This places the prevention/repair of collagen degradation as a key target for therapeutic agents.

ATRA has been shown to promote corneal regeneration, albeit weakly and by a mechanism that is as yet unclear. The development of selective RAR agonists that can be used to efficiently treat keratoconjunctival disorders and which are safe could have major benefits.

Kimura and colleagues have observed that the RARγ agonists R-677, CD-437 and BMS961 display a significant capacity to supress collagen type I degradation when tested against rabbit keratinocytes. These agonists were also found to be effective in supressing the collagen contraction during corneal cicatrisation and conjunctival cicatrisation [128].

6.7. Inflammatory Conditions

The precise mechanisms of alcohol induced liver disease (ALD) remain undetermined. However, the secretion of inflammatory cytokines, oxidative stress and lipid peroxidation toxicity of acetaldehyde are known to perpetuate an inflammatory reaction that results in cell apoptosis and tissue fibrosis [129-131]. The three stages of the disease include fatty liver disease (FLD), alcohol induced hepatitis (AIH), and finally liver cirrhosis [132]. While FLD is reversible with cessation of alcohol intake, inflammation in AIH leads to fibrosis and damage to areas normally involved in chemical detoxification, leading to irreversible scarring [133]. Finally, cirrhosis occurs often leading to the requirement for a liver transplant in severe cases. The prevalence of immune cells such as liver sinusoidal endothelial cells, Kupffer cells, dendritic cells, natural killer (NK) cells and natural killer T (NKT) cells in the liver [134] and the central role of inflammation in alcohol induced liver damage [135] lead to modulation of the immune response as an obvious therapeutic target in ALD. NKT cells are capable of promoting and suppressing the immune response and they represent an interesting means of regulating inflammation in ALD. In a 2014 patent, RAR selective retinoids and/or sulfatides were employed in a mouse model of ALD inflammation to supress the pro-inflammatory activity of the type 1 NK cells and to activate the type 2 NKT cells [136]. The retinoids tested as to their capacity to inhibit the activity of the type 1 NKT cells included AGN190168, AGN190129 ATRA, retinol, 9cRA, 13cRA, AM580, AC55649, CD-1530, etretinate and acitretin. The investigations showed that injection of isotretinoin during or before the period of alcohol consumption was protective of liver damage. In particular, mice

given a high dose of alcohol experienced increased levels of alanine transaminase (ALT), while mice given a high dose of alcohol and ATRA showed no elevation of ALT. Additionally, histological examination for steatohepatitis in the liver revealed no evidence of fatty liver disease in the mice given ATRA.

Osteoarthritis (OA) results from breakdown to the cartilage lining the joints and to the underlying bone. Inflammation of the synovium generally ensues which exacerbates the existing pain from bone grinding [137]. The effects of retinoids are known to contribute to pain [138] and retinoids are involved in the pathological processes of OA [139]. The use of antagonists of RARs has been proposed as a means to alleviate OA-associated pain. To avoid any potential toxic effects of pan-antagonists, the inventors of a new patent have used pyrazole analogs as RARγ selective antagonists to treat pain in OA [140]. Agonists were tested in the knee joints of rats in which the inflammatory agent monosodium iodoacetate had been administered resulting in an acute phase reaction. Using a capacitance test, the level of pain measured was found to be significantly inhibited in the rats treated with the analogues. The inventors may therefore have developed a method that could help alleviate pain generally, or specifically in osteoarthritis patients for whom other available treatments such as non-steroidal anti-inflammatory drugs have major side effects [141-143].

6.8. Muscle Damage

The causes of muscle injury are broad and common contributors include physical injury, infection, hypoxia due to vascular disruption and muscular dystrophy. Iwamoto and Pacifici have described the promotion of muscle repair by either the systemic or local administration of RARγ agonists, or by pre-treatment of stem cells with RARγ agonists prior to transplantation to the defect site [144]. The RARγ agonists were of the group consisting; CD-

271, CD-394, CD-437, CD-1530, CD-2247, BMS270394 and BMS189961. Muscle defects were generated in mice and the systemic or local administration of RARγ was performed at varying time points following the insult. The findings included that CD-1530 greatly increased repair to the defects, as compared to control mice in which the repair was largely fibrous scar tissue. Further support to the role of RARγ in muscle repair is that agonism of RARγ failed to promote acceleration of repair in γ-null mice. Of the RARγ agonists tested, BMS270394 was found to be the most effective. As to the stem cells, pre-treatment of mesenchymal stromal cells with a number of RARγ agonists was performed for periods of between 12 hours and 72 hours, and treated cells were administered at varying time points following muscle injury. Treatment on days 5, 7 and 10 following injury was found to be optimal for repair. The identification of treated mesenchymal stromal cells in the repair site was possible by DsRed labelling of the cells followed by fluorescent stereomicroscopy of histological sections. Overall it was found that local and systemic administration of the RARγ agonist BMS270394 enhanced muscle repair, as did mesenchymal stromal cells pre-treated with this agonist for 72 hours and cells administered between days 5 and 9 following injury.

7. Drug Composition and Delivery Methods

Appropriate delivery methods are essential to the therapeutic application of selective retinoids. A prominent feature of recent retinoid patents has been in regard to drug composition and administration. Traditionally retinoids have been delivered either topically or orally. While these delivery methods still dominate, alternative applications are being developed. One such example has been in relation to the use of expanded polytetrafluoroethylene (ePTFE) artificial grafts to treat critical limb ischaemia. A complication of stenosis is neointimal hyperplasia and Ameer and colleagues have developed a wrap or gel comprising of a biocompatible polymeric matrix and a selective retinoid which

is placed around a section of a vascular implant [145]. The retinoid diffuses through the implant and into the vascular wall and has been seen to stop the proliferation of smooth muscle cells and neointimal hyperplasia and up-regulate anti-thrombogenic genes and the production of nitric oxide. This represents a promising method of improving the failure rate of ePTFE grafts in critical limb ischemia, particularly when healthy autologous vein availability is limited.

Retinoids can cause skin irritation when used topically [146]. Formulations of retinoids contained within liposomes and solid lipid nanoparticles have been found to be clinically equivalent to retinoid gels and the side effects are significantly reduced [147,148]. Despite these results, issues with composition stability have limited their development. Patent [149] describes a novel composition of retinoids contained within microcapsules that reduces irritation and improves the chemical and physical stability of the composition. A double phase retinoid release was also achieved, with the benefit of a reduced initial exposure of the skin to retinoids and the maintenance of an effective therapeutic concentration. The employed retinoids included ATRA, 13cRA, acitretin, AGN191183, CD-271, AGN190168, retinal and etretinate. Another approach that has been used to reduce irritation is the incorporation of a retinoid within an aqueous gel suspension. Difficulties in preparing a homogeneous retinoid distribution have hindered the use of this technology. However, hydrophobic silica is suited to overcoming this limitation [150]. Hydrophobic silica was found to be chemically and physically stable and when tested on the flanks of Gottingen mini-pigs, Draize scale test results indicated that the composition was non-irritant.

Another invention describes a formulation of retinoids contained within oleosomes. These are oily liquid globules which are coated in a layer of lipophilic surfactant, hydrophilic surfactant

and an anionic surfactant [151]. Retinoids employed in the composition included 3″-tert-butyl-4′-(2-hydroxyethoxy)-4″-pyrrolidin-1-yl-[1,1′;3′,1″]-terphenyl-4-carboxylic acid. Following application of 20μl of the formulation to the ears of BALB/C mice for 7 days, indicators of inflammation were examined by clinical observation and measurement of the ear thickness. Additional testing of the various formulations included epidermal penetration kinetics. A chemically and physically stable slow release formulation, named composition 1, provided preferential localisation within the epidermis and controlled retinoid release, and importantly maintained a therapeutic concentration.

8. Expert Opinion

The successful use of ATRA to treat APL demonstrated for the first time differentiation therapy of a malignancy that was previously incurable. An overarching importance of this approach to treating cancer is the prospect of milder treatment, alone or when coupled with gentler chemotherapy, which is tolerated by very old patients. Twenty three percent of the UK population is projected to be aged $\geq$ 65 by 2034, older patients are often excluded from new and aggressive chemotherapy trials and common and intractable fears from toxicity often lead to no treatment in very old age as the preferred/advised option. Two decades of endeavours have still to make provisions of retinoid-based differentiation therapies for other cancers, particularly carcinomas that are prevalent or difficult to treat in old age. Current efforts to find a means of extending differentiation therapy are, therefore, important to meeting a 21st century societal need. As emphasised below, choosing the right synthetic retinoid and targeting the right malignancy, or other disorder, are crucial as different synthetic retinoids will be effective for different diseases.

Advances towards extending retinoid-based differentiation therapy to a variety of cancers focus on the investigation of novel synthetic retinoids that specifically agonise or antagonise a particular retinoid receptor subtype(s). However, highly specific and selective synthetic retinoids have been available for quite some time [152], including antagonists [152,153] and, in particular, a RARα subtype specific agonist [154]. In keeping, the use of novel synthetic retinoids as anti-cancer agents, as opposed to extending the use of ATRA, has been of interest for a decade, as brought to attention in 2001 by Altucci and Gronemyer [16]. In the case of APL, agonising RARα, as opposed to all RARs by ATRA, is sufficient to differentiate APL cells and the use of an agonist of RARα might well avoid ATRA-provoked retinoic acid syndrome which can be fatal. However, an agonist of RARα is very unlikely to replace the use of ATRA to treat APL. This therapy is highly successful and clinical trial of an RARα agonist would be restricted to a relatively small number of patients who have failed 1st and 2nd line treatments. Therefore, it is encouraging to see the use of retinoids to treat other cancers is being explored. The choice of the right synthetic retinoid is crucial as, for example, a RARγ agonist and baxarotene have been shown to have a beneficial effect against n4-NQO-induced oral epidermal cancers in mice and a hybrid molecule which selectively agonises RARβ and RARγ and inhibits histone deacetylase has been shown to be effective *in vitro* against breast cancer cell lines. Identifying the right RAR subtype-selective retinoid may provide new ways forward to differentiation therapy for malignancies other than APL.

ATRA has long been known to drive differentiation of keratinocytes. Administration of a retinoid topically provides a safe form of delivery and, for example, *Tazorac*® cream, containing the RARβγ agonist tazarotene, is a safe and effective treatment for adult and teen acne. An interesting way forward to the use of the localised release of retinoids is a patent that describes the benefit of placing a wrap/gel containing a selective retinoid around a

vascular implant to improve the success rate by preventing the proliferation of smooth muscle cells. Again the choice of synthetic analogue is crucial: agonizing RARγ is beneficial to muscle repair as a recent patent describes a method for muscle regeneration by means of local or systemic administration of a RARγ agonist. New methods of delivery and boosters to maximise activity, as describe in recent patents, are also important to these possible clinical developments.

Cancer and skin disorders have for quite a number of years been seen as targets for retinoid therapies. Albeit, retinoids are known to affect a wide variety of cell types and some recent patents extend the disease targets of interest in terms of retinoid-based therapies. Applications of retinoids to inflammatory, neurodegenerative, metabolic and ophthalmic disorders have been patented. The findings indicate a broadening of the possible therapeutic uses of retinoids. Moreover and to return to the theme of old age, retinoids may have a future in ensuring a health old age by preventing neurodegeneration and the shift in the immune system in older people towards an inflammatory profile, termed inflammaging. In this case, a chronic production of inflammatory cytokines appears to remodel the system. Whether this can be controlled by the systemic administration of an appropriate and safe retinoid is particularly interesting. All in all, the prospect of extending the current therapeutic uses of retinoids should be viewed as most promising in view of ongoing investigations of new synthetic analogues, the combined use of retinoids and other agents and a range of target clinical disorders.

Conflict of interest

The authors declare there are no conflicts of interest.

Acknowledgements

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Figure 1 Structures of selective synthetic ligands for retinoic acid receptors

(B) shows the structures of RAR selective agonists and antagonists employed in patents. For comparison the structures of ATRA, 9cRA and 13cRA (A) and RXR selective agonists (C) are shown.

Table 1 Tissue distribution of the RAR subtypes and isoforms.

RAR isoform	Tissue	Technique used	Organism
RARα1	*Overexpressed in* haematopoietic cell lines; *also expressed in:* kidney, prostate, spinal cord, cerebral cortex hepatoma-derived cell line, liver, spleen, uterus, ovary, haematopoietic cell lines	Northern Blot assay	human
RARα1	Skin – spinous and granular layer	Non-radioactive in situ hybridization	human
RARα1	Brain, skin, intestine, muscle, heart, lung, liver, kidney	Northern Blot assay	mouse
RARα2	Intestine, lung, liver	Northern Blot assay	mouse
RARβ1	Fetal (kidney, lung, skin) *Undetectable in* heart, colon, muscle, lung, spleen from adult	RNase protection assay	human
RARβ1'	ATRA-resistant human lung cancer cell lines	PCR, immunoblotting	human
RARβ2	epithelial tissues, cancer lung tissue neural tissue	RNAase protection assay, Southern blot analysis and Northern Blot assay	human
RARβ2	*High:* Kidney, prostate, spinal cord, cerebral cortex hepatoma-derived cell line *Average:* liver, spleen, uterus, ovary, *Undetectable in:* haematopoietic cell lines	Northern Blot assay	human
RARβ4	Breast cancer	Northern Blot assay, RT-PCR	human
RARβ5	normal, premalignant, and malignant breast epithelial cells	RT-PCR, Western Blot	human
RARγ	Skin – spinous and granular layer	Non-radioactive in situ hybridization	human
RARγ1	Skin - the predominant isoform	Northern Blot assay	mouse
RARγ2	Skin – very low level (<5%)	Northern Blot assay	mouse

Table 2 Examples of retinoid compounds employed in patents from 2014 to the present time.

Number & Patent	Chemical Name	Compound Name
US20140050676A1 "Method for treating skin with retinoids and retinoid boosters"	2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	AGN100335/Tretinoin (all-*trans*-retinoic acid), pan-RAR agonist
WO2015138354A1 "Combination therapy for head and neck cancer"	4-(6-Hydroxy-7-tricyclo[3.3.1.13,7]dec-1-yl-2-naphthalenyl)benzoic acid	CD-1530, RARγ agonist
US20140051760A1 "Hybrid molecule having mixed retinoic acid receptor agonism and histone deacetylase inhibitory properties"	6-(5,5,8,8-tetramethyl-6,7-dihydronaphthalen-2-yl)naphthalene-2-hydroxamic acid	SR3957/TTNN hybrid 3 molecule, RAR agonist
WO2014085494 "Combination therapeutics and methods for the treatment of neurodegenerative and other diseases"		A retinoid derived from the group; AGN100335, retinol, retinol acetate, retinol palmitate, 13-cis-retinoic acid, and LGD-1069
WO2014199905A1 "Medicament for treatment of alzheimer's disease"	4-[[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)amino]carbonyl]benzoic acid	Am80, RARα agonist
	4-(7,8,9,10-Tetrahydro-7,7,10,10-tetramethylbenzo[*b*]naphtho[2,3-*f*][1,4]thiazepin-12-yl-benzoic acid	HX630, RXR pan agonist
CN103561751 "Generation of neural stem cells from human trophoblast stem cells"	2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	AGN100335/Tretinoin (all-*trans*-retinoic acid), pan-RAR agonist
WO2014113695A1 "Methods of treating diseases associated with high fat diet and vitamin a deficiency using retinoic acid receptor agonists"	4-[4-(2-Butoxyethoxy-)-5-methyl-2-thiazolyl]-2-fluorobenzoic acid	AC261066, RARβ2 agonist
	4'-Octyl-[1,1'-biphenyl]-4-carboxylic acid	AC55649, RARβ agonist
	4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid	AM580, RARα agonist
US20140194517A1 "Treatment of graft-versus-host disease disorders using RAR antagonists"		RARα and/or RARγ antagonists (compounds not specified)
EP2918290A1 "Therapeutic agent for keratoconjunctive disorders"	(E)-4-(2-{3-[(1H-pyrazole-1-yl)methyl]-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalene-2-yl}vinyl)benzoic acid	R-667, RARγ agonist
	6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene acid	CD-437, RARγ agonist
	3-fluoro-4-[2-hydroxy-2-(5,5,8,8-tetramethyl-5,6,7, 8-tetrahydronaphthalene-2-yl)acetylamino]benzoic acid	BMS961, RARγ agonist
US20140187504A1 "Prevention and treatment of inflammatory conditions"	6-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzothiopyran-6-yl)ethynyl]-3-pyridinecarboxylic acid ethyl ester	AGN190168/Tazarotene, RARβγ agonist
US20140275049A1 "Substituted pyrazole analogues as RAR antagonists."		Compound names not specified, RARγ antagonists
US20140303223A1 "Composition and method for	3-Fluoro-4-[(R)-2-hydroxy-2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-naphthalen-	BMS270394, RARγ agonist

muscle repair and regeneration"	2-yl)-acetylamino]-benzoic acid	
	4-(6-Hydroxy-7-tricyclo[3.3.1.13,7]dec-1-yl-2-naphthalenyl)benzoic acid	CD-1530, RARγ agonist
	6-(4-Hydroxy-3-tricyclo[3.3.1.13,7]dec-1-ylphenyl)-2-naphthalenecarboxylic acid	CD-437, RARγ agonist
	6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid	CD-271, RARγ agonist
US201500719841"Controlled and localised release of retinoids to improve neointimal hyperplasia"	2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid	AGN100335/Tretinoin (all-*trans*-retinoic acid), pan-RAR agonist
US20150190372A1 "Microcapsules containing retinoids, method of preparing same, and pharmaceutical compositions containing same"	3″-tert-Butyl-4′-(2-hydroxyethoxy)-4″-pyrrolidin-1-yl-[1,1′;3′,1″]-terphenyl-4-carboxylic acid	Company name not specified, selectively activates RARγ relative to the subtypes α and β Protected in Patent Application WO2006066978
US20150150974A1 "Aqueous-gel-type topical compositions in the form of a homogenous suspension of an active principle of the class of retinoids containing at least one hydrophobic silica"	3″-tert-Butyl-4′-(2-hydroxyethoxy)-4″-pyrrolidin-1-yl-[1,1′;3′,1″]-terphenyl-4-carboxylic acid	Company name not specified, selectively activates RARγ relative to the subtypes α and β. Protected in Patent Application WO2006066978
US20150147403A1 "Dermatological composition comprising oleosomes and retinoids, process for preparing the same and use thereof"	3″-tert-butyl-4′-(2-hydroxy-ethoxy)-4″-pyrrolidin-1-yl-[1,1′;3,1″]-terphenyl-4-carboxylic acid	Company name not specified, selectively activates RARγ relative to the subtypes α and β. Protected in patent application WO2006066978

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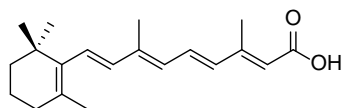
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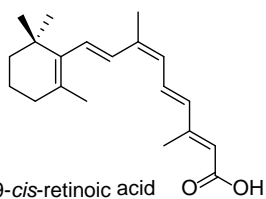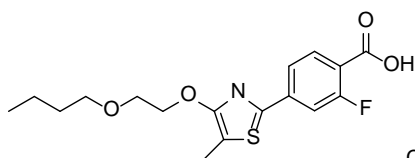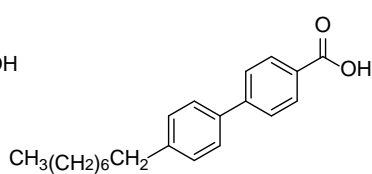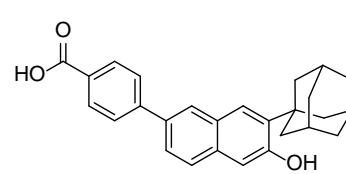
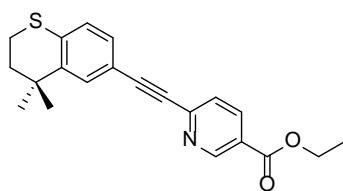2. 9-*cis*-retinoic acid
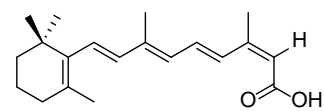
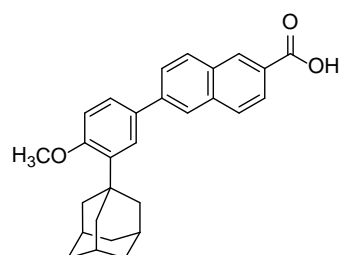3. 13-*cis*-retinoic acid
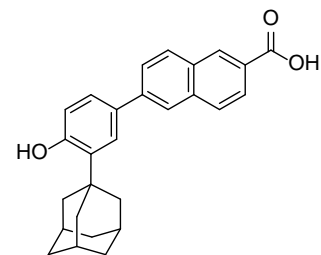
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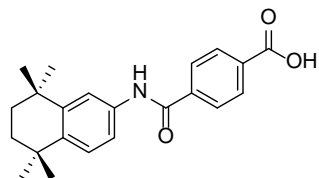
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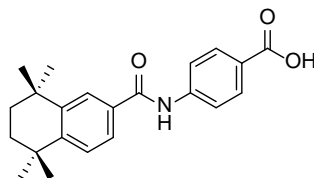
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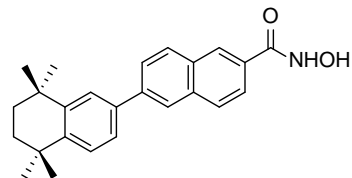
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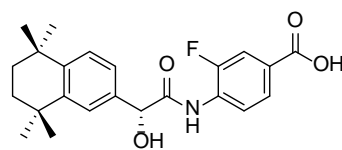
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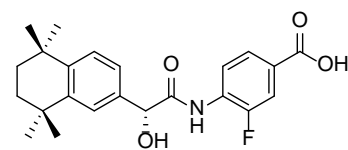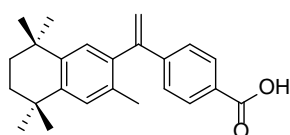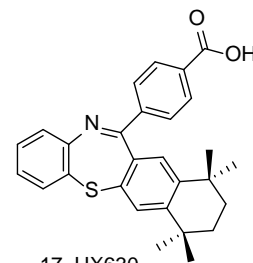
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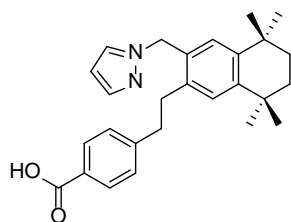
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