Ovarian cancer and KiSS-1 gene expression: A consideration of the use of Kisspeptin plus Kisspeptin aptamers in diagnostics and therapy.

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Abstract

Gynaecological cancers continue to present a significant health burden upon the health of the global female population. This deficit is most prominent with ovarian cancer which possesses the lowest survival rate compared to all other cancers occurring within this anatomical region, with an annual UK-mortality of 7,300. The poor tolerability and selectively of the treatment options that are currently available is likely to have contributed to this high mortality rate thus, demonstrating the need for the development of enhanced therapeutic approaches. Aptamer technology would involve the *engineering* of specifically sequenced oligonucleotide chains, which bind to macromolecular targets with a high degree of affinity and selectively. Recent *in-vitro* studies conducted upon the clinical utility of this technique have supported its superiority in targeting individual therapeutic drug targets compared to various other targeting moieties currently within therapeutic use such as, monoclonal antibodies. For this reason, the employment of this technique is likely to be favourable in reducing the incidence of non-specific, chemotherapy-associated adverse effects. Kisspeptin is a naturally expressed polypeptide with an established role in the development of the reproductive system and other proposed roles in influencing the ability of ovarian cancer growths to exhibit the metastasis hallmark. This distinctive feature would indicate the potential for the manipulation of this pathway through the application of aptamer structures in developing a novel prophylactic strategy and improve the long-term outcome for ovarian cancer patients.

Key words Aptamer . Oligonucleotide . Ovarian Cancer . Anti-cancer . Kisspeptin . KISS

1. Introduction

Ovarian cancer can result from various abnormal growth of tissues originated from the fallopian tubes, uterus, cervix, and the superior area of the vagina and not just from ovaries. The current epidemiological figures have demonstrated ovarian cancer to possess the lowest survival rate compared to all other gynaecological cancers, with the condition being estimated to possess an annual mortality of 184,799 deaths globally (Bray et al., 2018). Ovarian cancer has been classified as the 6th most common cancer affecting UK-resident females and is estimated to possess an annual national mortality and morbidity equating to 4,227 and 7,300 respectively (Office of National Statistics, 2018). It is also indicated as the second most common malignancy after breast cancer in women over the age of 40 particularly in developed countries (Vargas, 2014). The mortality rate has not significantly changed in the past 30 years. 90% of patients diagnosed at stage I and II have a 5- year survival rate of 90% and 80%, respectively and this is significantly reduced to 25% when diagnosed at stage III and IV. Due to the lack of an accurate and reliable screening tool and vague symptoms over 70% of ovarian cancers are only diagnosed at stage III and IV and less than half of patients survive after 5 years of diagnosis (National Cancer Institute, 2018; Chien and Poole, 2018; Bowtell and Christie, 2017; Bhoola and Hoskins, 2006). The risk of developing ovarian carcinomas increases with age and women are most at risk between the age of 50 and 70 years. Younger women under the age of 30 years rarely develop the disease (National Cancer Institute, 2018; American Cancer Society, 2018; Foong and Bolton, 2017). The objectives of this review article were to gain a greater insight into the therapies available for the treatment of ovarian cancers, and the potential benefits of considering the emerging evidence base surrounding aptamer technology and the Kisspeptin biochemical pathway in the development of new therapeutic strategies to improve the long-term outcomes of patients diagnosed with this condition.

2. Information sources:

The PubMed electronic database between 2004 and 2021 was consulted to gain an insight into the current advances of aptamer technology and the therapeutic potential of its use against ovarian cancer. In order to focus the effort upon evaluating the recent advances within this field, the literature search was restricted to only consider studies conducted within 2004 and 2021, with the exception of historical discoveries. This search was further expanded

through applying a manual process of reference analysis of each of the articles identified from the initial search.

3. Searching:

The electronic database search conducted had identified 42562 studies (PubMed/Medline) using the following search terms: (Aptamer OR Aptasensor OR Aptamer-targeted imaging OR targeted delivery OR aptamer-radiolabelling) AND (Cancer OR Kisspeptin OR ovarian cancer). Furthermore, an additional 94 information sources were individually identified through reference analysis and evidence-led investigation and thus, incorporated into this study. Through manually assessing the titles and abstracts of the studies initially identified, it had allowed for early exclusion of clearly irrelevant literature from this research. The eligibility of the remaining studies for incorporation into this research document were evaluated using the PRISMA checklist. A total of 42,533 studies were excluded from being incorporated into this systematic review and this led to 118 articles which were included in this review (**Fig.** 1). The vast majority of the studies incorporated into this review were conducted within North America and Europe.

4. Study Selection

The inclusion criteria were designed to assist in the identification of valid studies for consideration in regard to the research question prior to the application of the PRISMA checklist. This selection process involved assessing each study against 3 criteria. (1) Randomised controlled trails, systematic reviews, meta-analysis studies and observational (prospective and retrospective) studies; (2) Use of cancer-focused Kisspeptin and KiSS-1 expression studies; (3) Oligonucleotide aptamer studies used.

5. Background of ovarian cancer

5. Ovarian cancer

Approximately 5% of all ovarian cancers are derived from non-epithelial cells such as germ cells and sex-cord-stromal cells. Ovarian cancers derived from epithelial cells are the most

common type and can be divided into type 1 and type 2. Type 1 cancers consist of low-grade serous carcinoma, clear cell, mucinous and endometrioid, whilst type 2 cancers include high-grade serous carcinomas (HGSCs) and are the most common type comprising 70%-80% of all epithelial ovarian cancers (Kurman and Shih, 2016). Type I carcinomas are suggested to be caused by inflammation, endometriosis and continued ovulation cycles. Type I cancers have a good prognosis as they are confined to the uterus at the time of diagnosis and have a low rate of recurrence of approximately 20% (Morice et al., 2016). 5%-15% of all epithelial ovarian cancers are derived from endometriosis which have a better outcome than the types that are not originated from endometriosis. Type II tumours which are more associated with significantly higher mortality rates and are often diagnosed late with poor prognosis, generally affect older women, and have higher rates of recurrence of approximately 50% with much lower survival rate of 5 years (Morice et al., 2016). Type II tumours are linked to genetic mutations of genes such as BRCA and p53, RAD51D, RAD51C, BRIP1 and to a lesser extent HNPCC genes and certain transcription factors such as HOX, MYC, FOXM1, PAX8 and MECOM (Chen and Berek, 2018; Tung and Garber, 2018; Antoniou et al., 2003; Norquist et al., 2016; Geary et al., 2008; Watson et al., 2001, Nameki et al., 2021).

The employment of surgical, radiotherapeutic and chemotherapeutic techniques would constitute as the main strategies used to treat cancer (Sullivan et al., 2015; Cancer Research UK, 2018). The provision of carboplatin as a six-cycle drug therapy is recommended as a first-line treatment for patients diagnosed with stage I ovarian cancer growths, this treatment would be substituted with paclitaxel in more progressed cases (Stage II-IV) (NICE, 2011) (Table 1). An open-label, randomized phase 3 trial (n=692) had evaluated the efficacy of the paclitaxel drug therapy at a weekly and 3-weekly dosage had concluded that the progression-free survival rate achieved to be not statistically significant between each of the treatment regimens (Chan et al., 2016). In more advanced stages of the disease surgical debulking is followed by chemotherapy. Platinum doublet therapy with usually paclitaxel either intraperitoneally or intravenously for 6 cycles has been the standard care for years (Jelovac and Armstrong, 2011). Although in majority of the patients a complete clinical response is achievable however, the rates of recurrence are high and can vary depending on the stage of the disease. For example, patients with stage III and higher have an approximately 70% chance of recurrence within two years of diagnosis (Ozols et al., 2003). Re-treatment with the platinum- based doublet therapy will be employed if the symptoms recur. In patients resistant to platinum therapy recurrence happens before 6 months after the last dose of platinum treatment. The treatment options for these patients are pegylated liposomal doxorubicin, gemcitabine, topotecan or paclitaxel alone

or in combination with bevacizumab (Pujade-Lauraine, et al., 2014). Patients who initially go through debulking surgery followed by chemotherapy with positive clinical response might also go through maintenance therapy (Table 1). The current maintenance therapies include the use of targeted therapies such as poly(ADP-ribose) polymerase (PARP) inhibitors such as Olaparib, rucaparib, and niraparib, or angiogenesis inhibitors such as bevacizumab alone or in combination (Gonzalez-Martin et al., 2019; Lin et al., 2021; Gogineni et al., 2021). Although the targeted therapies recently approved as the effective maintenance therapies however, they are not devoid of adverse effects. For example, PARP inhibitors, which disrupt the DNA repair mechanisms, such as olaparib that was used in BRCA mutant platinum -sensitive advanced or high-grade ovarian cancer induced haematological abnormalities such as anaemia, neutropenia, and thrombocytopenia (LaFargue, et al., 2019). In addition, there was also a risk of developing myelodysplastic syndromes and acute myeloid leukaemia (AstraZeneca, 2020; GlaxoSmithKline, 2020).

Despite the therapeutic advantages gained through the inclusion of monoclonal antibodies in numerous oncological treatment guidelines, these benefits did not replicate across all cancer types; with a relatively poor level of efficacy being seen in patients suffering from gynaecological cancers (Burstein, 2005). This could have been due to the physiology of ovarian cancer cells compared to those of other cancer types, in which monoclonal antibody therapy has demonstrated greater success. It was known that a significant proportion of breast cancers are associated with an overexpression of the human epidermal growth factor receptor (HER2) protein and therefore, would be more susceptible to the actions of an anti-HER2 monoclonal antibody such as Trastuzumab, that was engineered to target and inhibit the HER2 receptor site (Burstein, 2005; Romond et al., 2005; Chames et al., 2009). With consideration of the mechanism of trastuzumab, it is clear that the efficacy of this therapy relies largely upon the cancer cells overexpressing the HER2 receptors to promote its further proliferation. This mechanism is likely to be responsible for the variation in the treatment's efficacy between both breast and ovarian cancer, as the HER2 receptor overexpression exhibited by each of these cancer types is seen to present in approximately 30% and 11% of the total cases respectively (Burstein, 2005; Mitri et al., 2012). This is supported by another study which found that trastuzumab failed to demonstrate a significant improvement in those suffering from ovarian cancer when used as a single agent and suggested that its efficacy may be enhanced in combination with other traditional chemotherapy agents (Mabuchi and Kimura , 2010).

However, some years later the FDA approved an angiogenesis inhibitor, bevacizumab, a monoclonal antibody, to the chemotherapy regimens in platinum-sensitive or resistant

recurrent ovarian carcinomas, as well as in the maintenance therapy which improved the progression-free survival in some phase III studies; although it did not significantly affect the overall survival (Pujade-Lauraine et al., 2014). However, some other phase III studies indicated an improvement in both the progression-free survival and overall survival (Coleman et al., 2017; Ruan et al., 2018).

In addition to the conflicting results associated with the beneficial effect of bevacizumab on overall survival from phase III clinical trials, it also became apparent that the use of this drug is also associated with some adverse effects such as hypertension, proteinuria, exfoliative dermatitis, renal haemorrhage. Furthermore, bevacizumab also increased the risk of development of non-gastrointestinal fistula formation, arterial thromboembolic events, and nephrotic syndrome and bowel perforation in ovarian cancer patients (Randall and Monk, 2010).

Another major drawback of the clinical use of monoclonal antibodies relates to their acceptability to the host's immune system and the risks of non-specific effects (Chames et al., 2009). Many of the murine-based antibodies synthesised at the initial stages of antibody development were observed to often stimulate the immune system of the patient shortly after their administration, leading to their quick destruction (Chames et al., 2009). Moreover, the necessary large-scale production of antibodies and extensive purification steps greatly contribute to their elevated production cost compared to conventional medicines. This requirement is coupled with the average effective dose of antibodies requiring 8-16 doses of $375 mgmL^{-2}$ to achieve clinical efficacy, causing the health costs incurred for each patient receiving monoclonal antibody therapy to be deemed unaffordable by many of the lower economic classes (Chames et al., 2009). In addition, the large size and relatively high molecular weight of antibodies compared to conventional drug compounds can become problematic when considering the accessibility of these proteins into deeper regions of tumour growths, a disadvantage which is known to be a contributor to the limited efficacy of this drug class.

In summary, the above studies have demonstrated the current advances and clinical applications of this field of targeted pharmacotherapy as well as, the various factors limiting its efficacy within practice. With consideration of this and the rising morbidity of cancer, it is demonstrated that there is a strong need for the development of more efficient, targeting techniques to assist in improving the safety, tolerability, and selectiveness of the current chemotherapeutic agents.

6. Significance of the Kisspeptin pathway

KiSS-1 was originally identified as a human malignant melanoma metastasis suppressor gene localised on chromosome 1q32.1 (Lee et al., 1996), that encoded for the 54 amino acid metastin peptide, which acted via the Kisspeptin receptor (KISS1R) to inhibit metastasis (Ohtaki et al., 2001). The term Kisspeptin is now used for the metastin peptide and its derivatives that bind to KISS1R, also known as GPR54 (Kotani et al., 2001; Pinilla et al., 2012). The Kisspetin peptides and the KISS1R plus associated mRNA are both found in the CNS (Gottsch et al., 2004; Kotani et al., 2001; Muir et al., 2001; Rometo et al., 2007). The biological activity of Kisspeptin is linked to the C-terminus of the 54 amino acid Kisspeptin, with shorter C-terminal fragments of 10, 13 and 14 amino acids (Kisspeptin-10, Kisspeptin-13 and Kisspeptin-14 respectively) retaining activity via the KISS1R to inhibit metastasis (Kotani et al., 2001; Pinilla et al., 2012).

The metabolism of the Kisspeptin by matrix metalloproteases (MMP) results in inactivation of the peptide in terms of its anti-metastatic action (Takino et al., 2003). However, recent studies have identified the Kissorphin peptides (Milton, 2012) which are Kisspeptin fragments that have lost the last 4 C-terminal residues required for KISS1R binding but are still able to activate both NPFF1 (GPR147) and NPFF2 (GPR74) receptors and exert biological actions (Milton 2012; Gibula-Tarlowska et al., 2017; Gibula-Tarlowska et al., 2019a; Gibula-Tarlowska et al., 2019b; Gibula-Tarlowska and Kotlinska, 2020). The generation of Kissorphins could be mediated via MMP processing of Kisspeptins. Some of the biological actions of Kissorphin peptides are shared with via the NPFF1 and NPFF2 receptors natural ligand Neuropeptide-FF (NPFF) and Kisspeptin peptides (Lyubimov et al., 2010; Oishi et al., 2010; Milton, 2012; Elhabazi et al., 2013). These actions of Kisspeptins and their metabolites the Kissorphins may be relevant to therapeutic use of Kisspeptins in an ovarian cancer setting. Similarly, studies have shown that antibodies against Kisspeptin can cross-react with NPFF (Iijima et al., 2011; Chilumuri et al., 2013), raising the possibility that immunoassay measurement of Kisspeptin could be influenced by natural NPFF levels. Immunoassays used to measure Kisspeptin 10 (Milton, et al., 2012) are known to use the same antibody as used by Chilumuri et al., (2013) and were shown to cross react synthetic NPFF plus Kissorphin derivatives. As such immunoassay determination of Kisspeptin as a potential marker in an ovarian cancer setting may not always reflect biologically active material and therefore not always be fully representative of the anti-metastatic activity of Kisspeptin present in patient samples.

Kisspeptin is a central regulator of the reproductive axis via stimulation of gonadotropin releasing hormone release (Holly et al., 2015). Depletion of either KiSS-1, Kisspeptins or the KISS1R is associated with several forms of infertility, such as isolated hypogonadotropic hypogonadism (IHH) and central precocious puberty (CPP) (Chan, 2013; Brioude et al., 2013; Gottsch et al., 2009; Miraoui et al., 2013). Mutations within the KiSS-1 and KISS1R genes also contribute to the presentation of either IHH or CPP (Lanfranco et al., 2005 ; Pallais et al., 2006). Kisspeptin influences the progression of several diseases including, Alzheimer's disease, Polycystic ovary syndrome (PCOS) and specific types of cancer (Vincenza et al., 2018; Milton et al., 2012; Murphy and LeVine, 2010). PCOS is linked to a range of metabolic symptoms and associated with changes in the reproductive hormones activated by kisspeptin (Kotani et al., 2001; Holly et al., 2015; Legro et al., 1999; Glueck et al., 2003; Dunaif et al.,1989; Elting et al., 2001; Holte et al., 1996; Diamanti-Kandarakis et al., 2007; Talbott et al., 2000; Valkenburg et al., 2008). Kisspeptin has a pathophysiological role in PCOS and may be a useful biomarker (De Assis Rodrigues et al., 2019; Yilmaz et al., 2014).

The Kisspeptin peptides influence the incidence of metastasis in several cancer types, including bladder, gastric and ovarian cancers (Beck and Welch, 2010; Ciaramella et al., 2018). Downregulation of KiSS-1 gene expression is associated with greater risk of distant metastasis in gastric cancer (Dhar et al., 2004) and has been demonstrated in more severe forms of bladder cancer (Sanchez-Carbayo et al., 2003). There are therefore suggestions that the KiSS-1 and KISS1R are potential targets for treatment of metastatic cancer (Vincenza et al., 2018). The anti-metastatic role of Kisspeptin involves an inhibitory effect upon cell invasion, motility and adhesion; all of which play key roles in the embryonic development thus, potentially influencing the reproductive potential of a female (Lee et al., 1996; Trevisan et al., 2018; Ohtaki et al., 2001). This proposition is further supported by the expression of both KiSS-1 and KISS1R genes at the ovary, ovarian duct and uterus, and the presence of significantly higher concentrations of Kisspeptin within pregnant females (Jayasena et al., 2014). The KiSS-1 gene mediates an anti-metastatic effect on ovarian cancerous tumours via a direct-action inhibiting protein kinase C in addition to inhibiting the detachment and migration of cancerous cells that are morphologically-adapted to undertake the epithelial-mesenchymal transition dissemination process (Jiang et al., 2005; Ciaramella et al., 2018). A clinical study investigating the association between the expression of a gene product of the KiSS-1 gene (Kisspeptin-54) and the prognosis of patients diagnosed with ovarian cancer had found patients expressing the greater levels of polypeptide macromolecule to possess a statistically significant reduction of presenting with microscopic residual tumours following surgical resection procedures

(p=0.0084) (Stafford et al., 2007). A promising proposition in manipulating this biological pathway involved the exogenous administration of Kisspeptin-54. Despite the preliminary data not associating this intervention with the production of significant adverse effects, the reduced expression of the complimentary G-protein coupled receptor of this macromolecule (KISS1R) by the majority of cancerous growths was found to present a challenge in preserving the efficacy of this supplementary therapy *in-vivo* (Vincenza et al., 2018; Beck and Welch, 2010).

The increased expression of MMP's within cancerous growths not expressing the KiSS-1 gene could contribute to tissue invasion and metastasis (Hata et al., 2007; Zucker et al., 1993). The relationship between MMP and KiSS-1 expression has also been observed with the development of liver metastasis originating from colorectal cancer types with low KiSS-1 expression in patients with elevated MMP-9 (Nomura et al., 1995). Earlier evidence surrounding this interaction had also eluded the role of inhibiting MMP-2 and MMP-9, which would potentially prevent inactivation of the Kisspeptin, preserving the anti-metastatic effects (Zhu et al., 2015). The use of Kisspeptin as a novel biomarker in monitoring the development of tumour metastasis within patients has been suggested for ovarian cancer (Chen et al., 2016; Prentice et al., 2007), however the crossreactivity of inactive MMP processed forms in immunoassays needs to be overcome (Chilumuri et al., 2013; Milton, et al., 2012).

7. Aptamer technology

The use of aptamer technologies provides a potential solution to the cross-reactivity issues with antibodies plus a potential route to develop both diagnostic and therapeutic compounds. Nucleic acid aptamers are single stranded DNA- or RNA-based oligonucleotide structures that possess an ability to bind to their complementary target with a great degree of affinity and specificity (Famulok and Mayer, 2014; Garst et al., 2011; Ku et al., 2015; Lakhin et al., 2013; Ellington and Szostak, 1990; Tuerk and Gold, 1990). Using libraries of short random nucleic acid sequences combined with selection assays allows the identification of aptamers sequences that are specific for a target which could be used in both diagnostic and therapeutic settings. The main methods for doing this involve the application of the Systematic Evolution of Ligands by EXponential enrichment (SELEX) process (Hori et al., 2018). Using a library with fixed primer regions at the 5' and 3' ends of the random sequences, the target protein can then be used to pull down binding aptamers which can then be amplified through the repeated application of the Polymerase Chain Reaction PCR) (Darmostuk et al., 2015). This process would allow the selection of specific Kisspeptin binding aptamers (**Fig. 2**). A range of selection

processes can be employed so for example with raising KISS1R specific aptamers it may be necessary to use Whole Cell SELEX, which would utilise the KISS1R target proteins present upon the membrane of living cells within an environment that resembles the structure *in-vivo* to a greater extent (Chauveau et al., 2007; Liu et al., 2009; Ohuchi, 2012). Aptamers can also be used in the development of sensors for disease monitoring, detection of pollutants and the drug discovery process (Bhalla et al., 2016). Aptamer based biosensors or 'aptasensors' are known to be superior in their accuracy compared to those employing monoclonal antibodies, due conformational changes during the binding of the aptamer to the target (Morita et al., 2018). There are many different types of aptasensors used within clinical study with one of the most notable involving the incorporation of an electrochemical molecule. This technique involved chemically bonding an aptamer bioreceptor to an electrode surface to which an analyte would be presented and retained. Following this, the aptamer-analyte complex would be exposed to a secondary aptamer molecule that would be targeted to bind to a different site on the surface of the analyte thus, producing an aptamer-analyte-aptamer complex often termed as an 'Aptamer sandwich' (**Fig. 3**). The secondary aptamer molecule is often conjugated with another charged species such as, gold nanoparticles, which would be detected by the electrode (Pavlov et al., 2004). Earlier detection of ovarian cancer growths using aptasensors has shown the strength of this technology in the ovarian cancer diagnostic field for the detection of CA125 (Hamd-Ghadareh et al., 2017) and there is the potential to apply similar strategies for detection of Kisppeptin or the KISS1R.

The use of aptamers for both diagnostic and therapeutic purposes would require the consideration of several factors such as, the relatively short shelf life, chemical stability and delivery strategies to ensure the aptamers are able to reach their molecular target. Currently, the use of base and backbone modification of the aptamer structure have demonstrated positive results in improving the stability of aptamer molecules thus, allowing them to become practical within a healthcare setting (Hori et al., 2018; Famulok et al., 2007; Hassanzadeh et al., 2018). Aptamer delivery into the central nervous system can also be achieved through the application of various techniques including, the entrapment of aptamer molecules into liposomes and protein transduction domains, and the manipulation of nanoparticles (Cheng et al., 2013; Koffie et al., 2011), this has been used with aptamers against targets relevant to Alzheimer's disease (Huiyu et al., 2015).

The use of aptamers has also displayed promise in optimising the current anti-cancer treatments through providing a potentially novel route in delivering these chemotherapeutic agents to cancerous growths at a great selectively and more cost-effective manner compared to

other targeting moieties that are currently used within practice notably, monoclonal antibodies. As mentioned previously, aptamer molecules possess a much higher degree of specificity in their binding to a molecular target compared to other targeting moieties. When coupled with the reduced cost incurred in aptamer production and the wide acceptability of targets ranging from small chemical mediators to entire cells, it is clear that their use within biosensor technology would be advantageous (Hori et al., 2018). A recent review of aptamers for use in ovarian cancer diagnosis and treatment has highlighted aptamers to known cancer markers such as CA125, CA70 plus HER2 for potential therapeutic use and CA125 for potential diagnostic use (Ruan & Li, 2021).

8. Strategies

Kisspeptin and KISS1R for ovarian cancer therapeutic plus diagnostic targeting are viable options based on the observations for the biological activities of Kisspeptin in relation to inhibitory effects on metastasis via activation of KISS1R. The multiple forms of Kisspeptins (Kotani et al., 2001; Pinilla et al., 2012) and the cross-reactivity of other peptides plus inactive metabolites (Iijima et al., 2011; Milton et al 2012; Milton, 2012; Chilumuri et al., 2013) cause potential problems in using immunoassays for diagnosis or therapy monitoring. A solution to this may be to use aptamer technologies and select specific aptamers with both positive and negative selection strategies to effectively identify Kisspeptin specific aptamers that are suitable for diagnostic use. It is clear from recent studies that diagnosis is likely to need multiple measures (Menon et al., 2021) rather than just Kisspeptin levels, for example a combination of Kisspeptin with known tumour markers (Chen et al., 2016).

The pharmacokinetic and pharmacodynamic properties of Kisspeptin and synthetic KISS1R agonists in humans (Abbara et al., 2020) raise the possibility of using such compounds in an ovarian cancer setting. Aptamers that act as receptor ligands show potential in cancer therapy (Zhang et al., 2021). Specific aptamers against the KISS1R may also offer potential and may help to rule out biological actions at other receptors such as the NPFF1 and NPFF2 which respond to Kisspeptin. Allosteric aptamers have been shown to enhance receptor activation by insulin (Yunn et al., 2021) and this approach may also provide a mechanism to enhance Kisspeptin activity. Peptide aptamers with structural similarities to Kisspeptin may also offer potential in this setting. A major advantage of using Kisspeptin itself is that it is an endogenous peptide and therefore less likely to be seen as foreign by the body and trigger an unwanted immune response. A disadvantage of Kisspeptin is the observation of desensitization

of KISS1R by repeated administration of Kisspeptin 10 (Seminara et al., 2006) and there is evidence of KISS1R downregulation in some cancer settings (Ly et al., 2020). There is also evidence of antimetastatic actions of Kisspeptin that are independent of KISS1R and these areas require further study (Ly et al., 2020).

The use of specific aptamers which have a high affinity for the Kisspeptin would allow for the more selective targeting of this molecular target within cancer pathophysiological mechanisms and reduce the incidence of a non-specific blockade of other chemical mediators or the blockade of Kisspeptin in a different signalling pathway, each of which could facilitate the production of adverse effects within the patient. For this reason, their application in ovarian cancer therapeutic strategies would be largely advantageous in the clinical management of these conditions and warrants further research.

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N.G.N.M. is named as the inventor on a UK patent held by the University of Roehampton for the use of kissorphin peptides to treat Alzheimer's disease, Creutzfeldt-Jakob disease or diabetes mellitus (Publication Numbers: GB2493313 B); under the University of Roehampton rules he could benefit financially if the patent is commercially exploited. N.G.N.M. is also a shareholder and director of NeuroDelta Ltd (Company No: 06222473; https://www.bioinformatics-protocols.com/Neurodelta/). This does not alter our adherence to the journal policies. The reference for this patent is: Milton, N. (2017) Kissorphin peptides for use in the treatment of Alzheimer's disease, Creutzfeldt-Jakob disease or diabetes mellitus. United Kingdom Patent Publication Number GB 2493313 (B).

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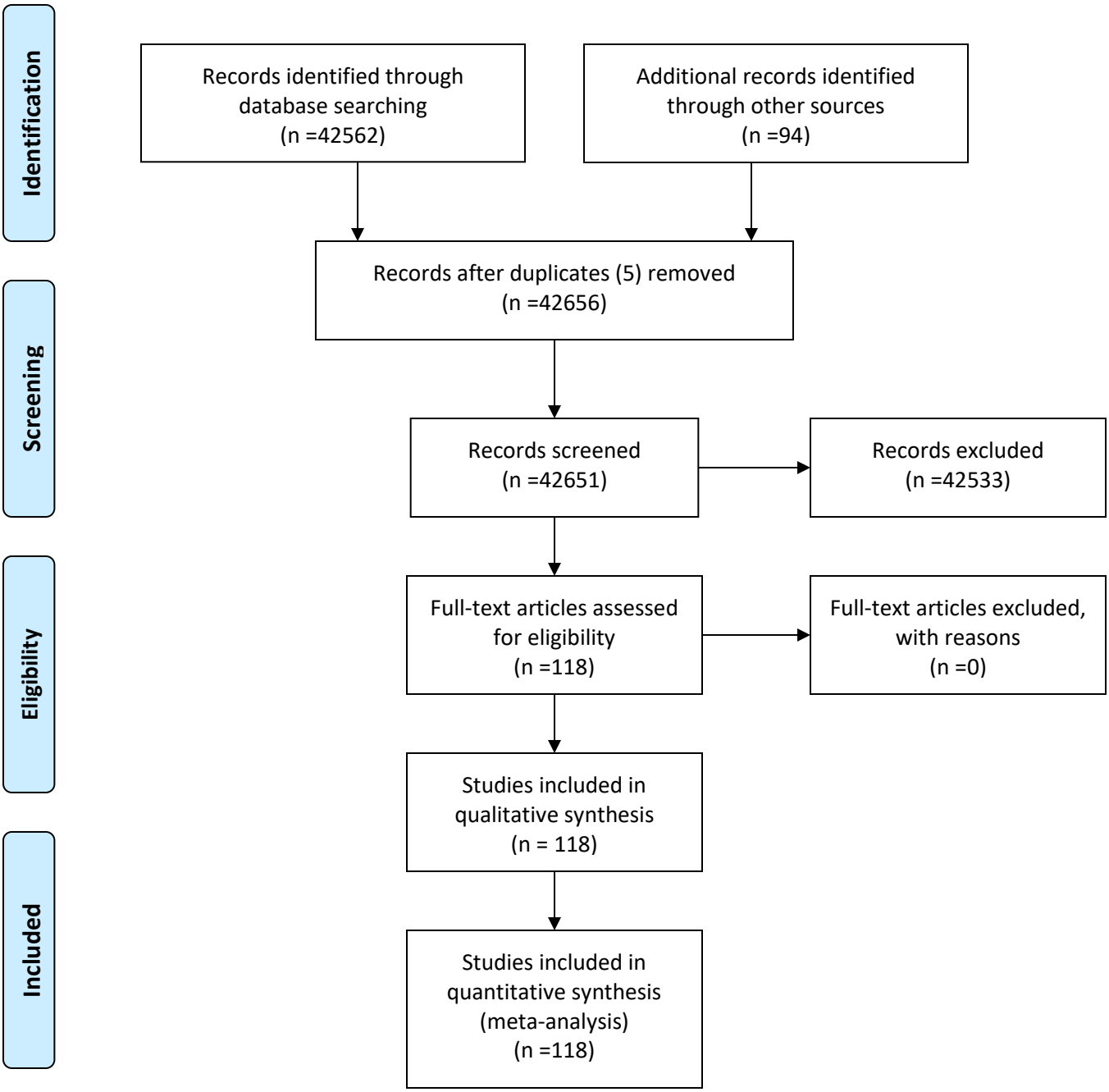
Table 1. Recommended strategies for the treatment of ovarian carcinomas.

Fig 1. PRISMA diagram for the screening and selection of studies.

Fig 2. A schematic diagram of the SELEX procedures used to isolate oligonucleotide sequences specific to a desired molecular target from an entire DNA library

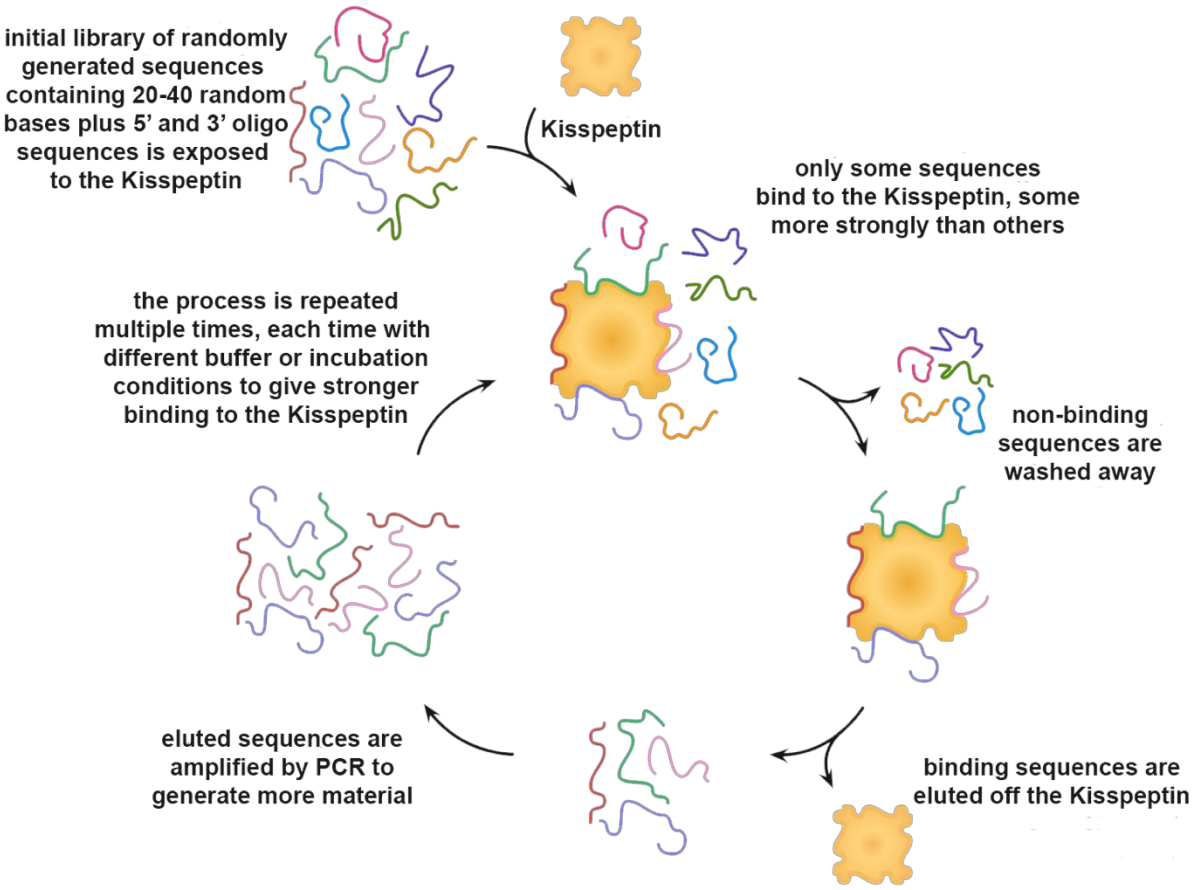
Fig 3. A labelled diagram depicting the sequence involved in the production of an aptamer-analyte-aptamer complex. (1) Analyte approaches the primary aptamer-electrode complex, (2) Analyte binds to the primary aptamer-electrode complex, (3) The secondary aptamer labelled with an ionised molecule approaches the electrode-primary aptamer- analyte structure, (4) The secondary aptamer binds to the analyte

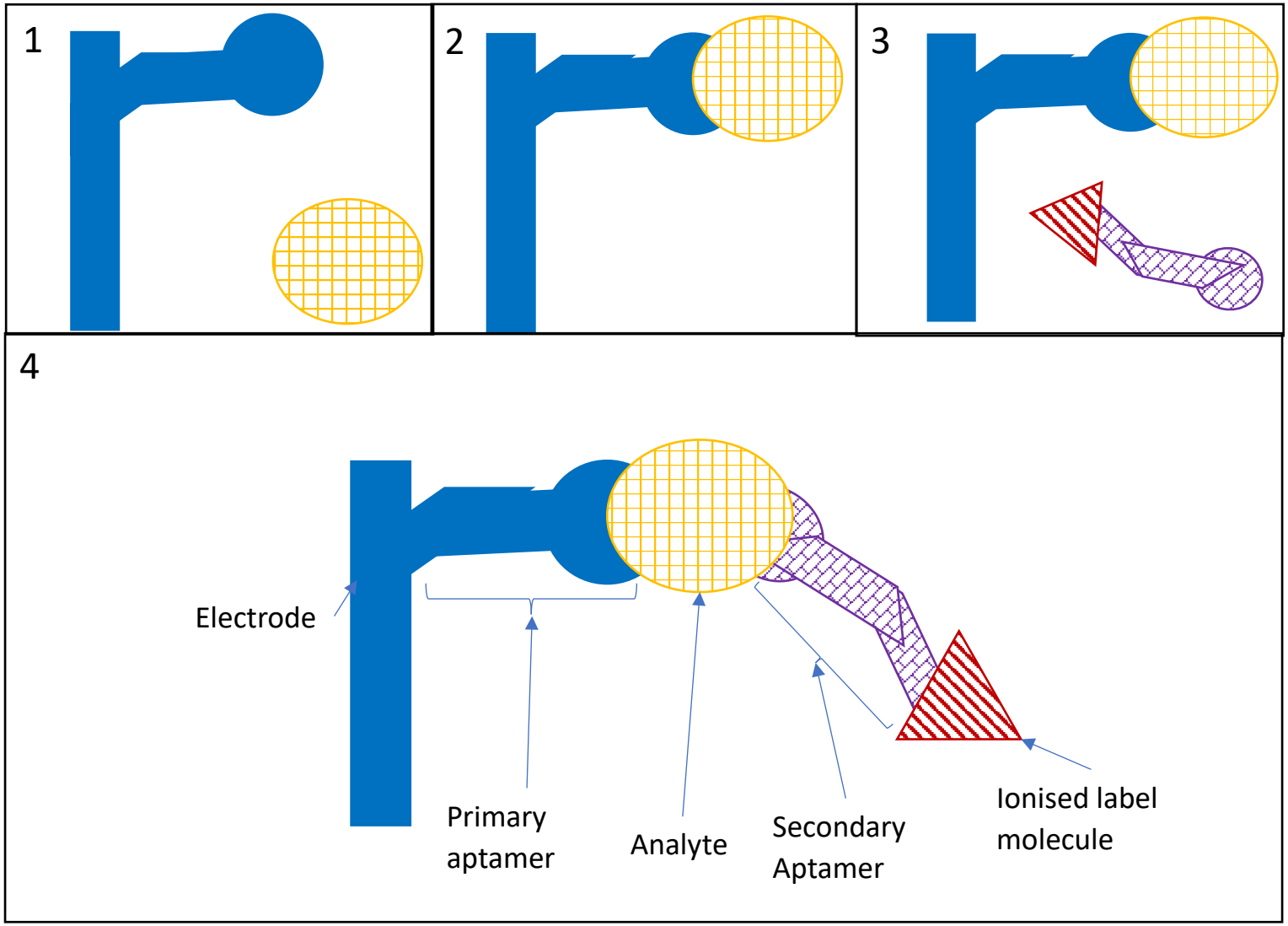
PRISMA 2009 Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.


initial library of randomly generated sequences containing 20-40 random bases plus 5' and 3' oligo sequences is exposed to the Kisspeptin
Kisspeptin
only some sequences bind to the Kisspeptin, some more strongly than others
non-binding sequences are washed away
binding sequences are eluted off the Kisspeptin
eluted sequences are amplified by PCR to generate more material
the process is repeated multiple times, each time with different buffer or incubation conditions to give stronger binding to the Kisspeptin


1
2
3
4
Electrode
Primary aptamer
Analyte
Secondary Aptamer
Ionised label molecule