1. Depression

Mental pain is less dramatic than physical pain, but it is more common and also harder to bear. The frequent attempt to conceal mental pain increases the burden: it is easier to say "My tooth is aching" than to say "My heart is broken."

Major depressive disorders (MDD), normally known as depression is a serious mental disorder of mammoth social and clinical relevance (1). The frequency of occurrence in lifetime is around 20% with the normal frequency globally of 5:2 women to men ratio (2). Moreover, twin studies depending on heritability show 50 – 60 % chances of depression during lifespan (3). Studies in adopted children also provide some insight into involvement of genes in depression showing less chances of disease progression. The role of heredity is clear for depression but we can't neglect the effects of environmental factors include early childhood trauma and stress (4).

Depressive disorders can be classified as shown in figure 1.1

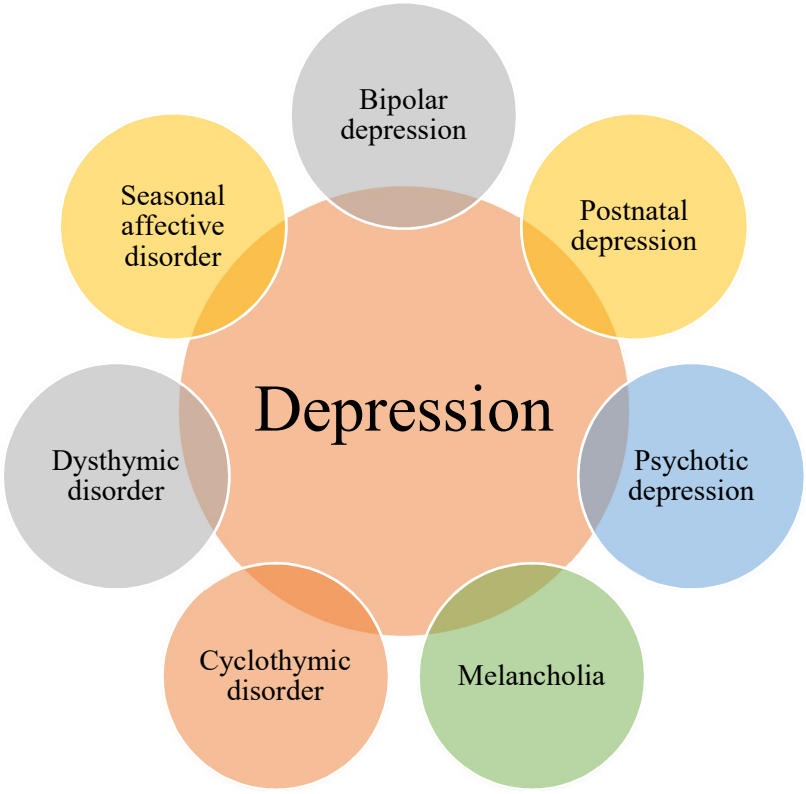

Figure 1. 1 Classification of depressive disorders

Symptoms commonly present in depressed person are shown in figure 1.2.

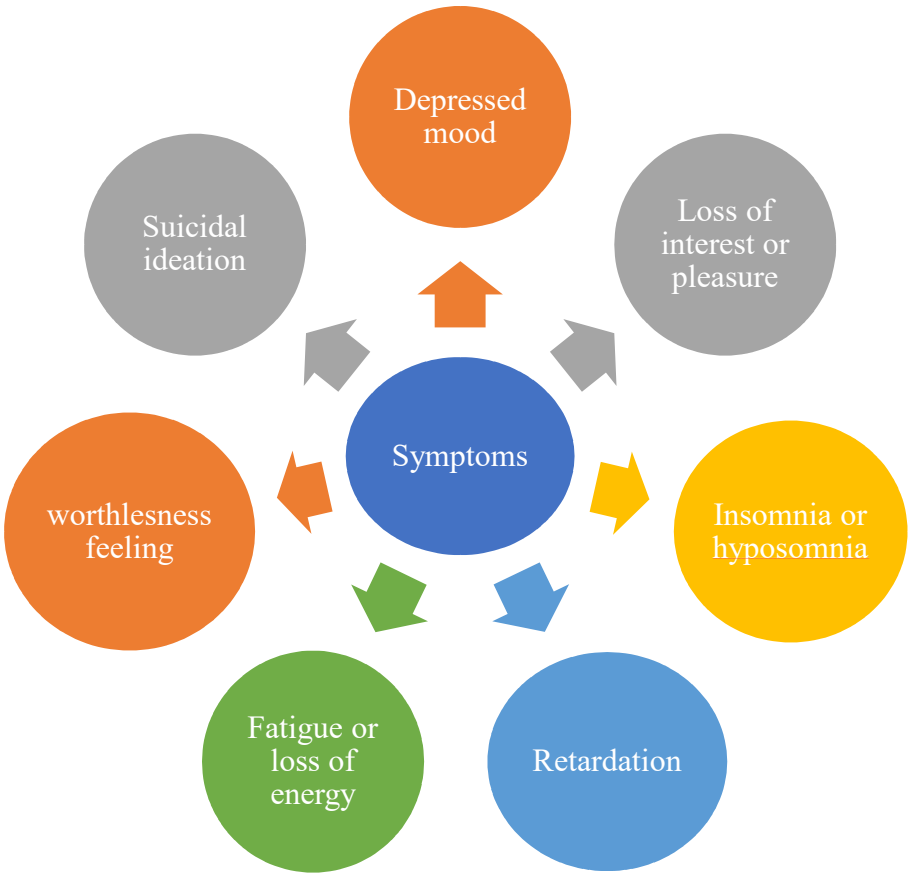

Figure 1. 2 Symptoms of depression

1.1 Medications for depression

Drugs that are used clinically for depression, mostly based on monoamine theory. Various hypothesis for depression include monoamine hypothesis, HPA axis hypothesis and Neurotrophin hypothesis of depression. Medications for depression can be classified as mentioned below:

- MonoAmine Oxidase inhibitors (MAOIs);

  Generic and brand names: Phenelzine (Nardil), Tranylcypromine (parnate), Isocarboxazid (marplan), Selegiline (emsam)

- Tricyclic antidepressants

  Generic and brand names: Amitrypriline (tryptizol), Clomipramine (anafranil), Imipramine (tofranil), Nortriptyline (allegron)

- Selective serotonin reuptake inhibiotors (SSRIs);

  Generic and brand name: Fluoxetine (Prozac), Sertraline (Zoloft), Citalopram (celexa), Escitlopram (lexapro)

- Atypical Anti-Depressants:

Generic and brand name: Bupropion (wellbutrin), Duloxetine (cymbalta), Mirtazepine (remeron), Trazodone (desyrel)

1.1.1 Limitations of Current Therapies

- Low remission rates

It is shown that by current medication treatment remission rate around only 40-60 %, it is indicating that there is need of new medications for the treatment of disease(5).

- High rate of recurrence

In patients who do recover, there is a high rate of recurrence and it has been found that approximately 75% of patients experience more than one episode of major depression within 10 years (6).

- Non-adherence to therapy

It is widely believed that antidepressants generally show only a slow onset of efficacy, often taking up to 6 weeks for maximum effect, in spite of rapid pharmacological actions (7). Such a delay may promote non-adherence to therapy and increase the risk of suicide early in treatment.

- Side effects

Side effects profile is also high as the treatment continues for a long time. SSRI class of drugs having side effects related to sexual dysfunctioning, insomnia and feeling of sickness(8). Tricyclic antidepressants and MAOIs are relatively more toxic than SSRI class of drugs(9). They sometime cause problem related to blood pressure, chest pain, severe migraine, abnormal fast heart beats.

- Drug – drug interaction is common due to long term therapy

Most of the antidepressants especially SSRI class of drugs inhibits the enzyme CYP450(10). This CYP450 is important in metabolizing many drugs due to long treatment of depression it is possible for a patient to take some other medication for any other complications and these drugs may alter the pharmacokinetic profile of other drugs (11).

None of the traditional treatments of depression are particularly effective (12). This does not mean that we should not keep using them, but it highlights the fact that new research and new insights into the treatment of depression are needed. Treatment efficacy for anti-depressants is relatively low with only about 30% of patients experiencing remission, and 30 to 40% of patients not showing any significant response (13). Moreover, anti-depressants, when they work, take several weeks or months before there is any response.

There is therefore an important need to develop therapeutic strategies devoid of side effects and capable of curing depression within a few days.

Regardless of the point that pharmaceutical firms continue lingering on the idea of "imbalanced chemicals in brain" as main reason for depression, this is an immense generalisation of an extremely more complex and serious condition. This imbalance of chemicals can be one of the many principal causative factors for depression but what results in chemicals (herein more specifically neurotransmitters) to become unproportioned is the main puzzle to be solved. Undoubtedly lifestyle of an individual and food habits or nutritional intake can impact the transmission of these chemicals and encourage constructive or destructive epigenetic expression, but they have not ability to correct the genetic mutations to its original form. Often depressive patients are said to have faulty genes that disturb process chain including: activation of neural path, neurotransmission, level of hormones and stimulation. As a result of gene favouring good mood, a very few individual hits the lottery of inheritance, they are inclined to have a tendency to feel happy, a happy go lucky type of persons, even if they don't know why. It is definitely not handy to spend life accusing ones genes if they are depressed, aqs one can accomplish nothing by complaining and only stimulates the feeling of "learned helplessness." Nevertheless, supposing to continue therapeutic involvements without any kind of symptomatic relief – is an indirect proof that genes may be holding you back resulting in one revolutionary intervention for depression that may be fully developed within the next few years is "gene therapy".

1.2 Role of gene therapy

A scientific practise to alter genes or their expression to avert various diseases can be called as "gene therapy". In the future, it is thoughtful for the medical professionals to cure depression using specific gene responsible for it. Theoretically it works by targeting faulty gene that results in depressive behaviour of patients using replacement with more favourable genes.

This gene therapy technique procedures through vector to facilitate a gene to specific cells only where the gene modification is required. After the successful insertion of gene, the gene is handled by the cells followed by synthesis of proteins. In this way synthesised or produced proteins follow the orders of specific genes within cells as dictated in programming of our body. For the specific treatment of depression using gene therapy

approach patients may require to replace mutated gene or protein manufacturing control or addition of a completely new gene.

- **Replacement of genetic mutations**: One tactic of using gene treatment is to replace a mutation in gene (accountable for depression) with a healthy copy of the gene that can cure depression.
- **Protein manufacturing control**: In few cases, gene therapy can be used to fundamentally deactivate definite genes overproducing causative proteins responsible for the depressive symptoms. Inhibition therapy like RNAi for depression inhibit production of specific proteins responsible for depression, gene therapy could lastingly change the over production of proteins.
- **Adding a new gene**: Somebody with depressive disorder may be deficient of a particular gene related to moderate to severe depression. A completely new gene or replicate or that defective gene could be entered into the body of such patients to cure symptoms related to depression.

1.3 Probable advantages of Gene Therapy

Numerous possible advantages of gene therapy are there, for the efficient management of depression. The most noticeable benefit is that it could be used fundamentally as DNA vaccination to avoid depression to forever occur in assured patients, and everlastingly cure depression in others. Gene therapy is able to correct genetic malfunctionalities at the root rather than assist as a temporary solution like pharmaceutical antidepressants.

- **Genetically engineered prevention**: In the near future, there are chances that depression could be cured with genetically engineered embryos. A few may not like the impression due to ethics "playing god" which may help a person to avoid dealing with a lifespan debility of depression. While some would debate that the application of genetic engineering well before the birth might be of considerable advantage to the treated patients along with the rest of society.

- **Individualized targeting**: If scientists can isolate definite genetic malfunctionalities accountable for actual depression, they may find individual dissimilarities. Two patients suffering from depression may have diverse genetic abnormalities accountable for the disease. As a result, gene therapy, in future, may be used for individual patients to cure the deformities and improve the performance of an individual.

- **Minimal side effects**: In the near future, treatment with gene therapy will be advanced and have negligible possibility of major side effects. Many patients with any form of depression hatred to take antidepressant medications because of their side effects. With this gene therapy, such adverse effects related with consumption of pharmaceutical antidepressants won't happen.

- **Permanent cure**: Some researchers firmly consider the gene therapy to ultimately improve all mental conditions, perhaps helping as a permanent cure. Addition of an adjusted copy of a problematic gene may regulate the neural functioning. This is not the same as regular treatment with pharmaceutical antidepressants which may alleviate a few symptoms of depression, but not able to cure underlying genetic problems.

1.4 Potential Pitfalls of Gene Therapy for Depression

Regardless of the fact that the treatment with gene therapy seems to be a new therapeutic perception for depression, however there are certain apprehensions related to the procedure. Definitely, treatment using therapeutic gene is yet to be evaluated as a non-toxic and safe option for human trials among those with depression. The efficacy of the technique isn't well-established, and the longstanding effects persist to be undetermined.

- **Cost**: Right now, the expenditures related to gene therapy would make it excessive costly for most patients suffering from major depression. Numerous gene therapy interferences may charge well over $1.5 million per patient. Till costs are not lowered, although the safety is recognized, and efficacy is verified – gene therapy still remains to be an unrealistic approach.
- **Delivery methods**: It is still a question as how to deliver gene for depression treatment. Supposing the genes were introduced to the viral vectors, it is significant to study the jeopardy of inflammations and toxicity. Researchers needs to create the flawless gene delivery, instituting both safety and therapeutic efficacy well before it can be verified and then used in humans.
- **Efficacy**: At present, it is not known for now to how much effective gene therapy would be for the effective treatment of depression in suffering patients. Though it sounds encouraging to target specific and precise genes to cure depression, the degree of therapeutic advantage remains uncertain. Supposing the genes are delivered through viral vector and more than one treatment is essential, the immune system of our body may acquire to attack the foreign particles rather

quickly – thus reducing the efficacy of gene therapy with successive administration.

- **Ethical concerns**: There is a range of ethical concerns that a certain class of society have with the use of gene as a part of treatment regimen. A few people may use them for deliberate enhancement of mood, while others can use them to treat depression. Some wealthy patients may perceive this costly gene therapy as an option with partial or no accessibility to the rest. These moral distresses need to be spoken for gene therapy to appear as a feasible treatment for any kind of depression.
- **Safety concerns**: Initial indications recommend that treatment with gene therapy may pose noteworthy safety concerns for the patients receiving them. Upon administration of new DNA, many side effects may be from mutations can occur. This may affect growth of tumour, leukemia, or end up in death. While some gene therapy appears as safe grounded on clinical trials, still there are some undoubtedly health risks to be considered.
- **Temporary results**: For gene therapy to assist as a "permanent remedy," the DNA inserted target cells need to be alive for the long time. The cells in which DNA is administered, must be steady, or else it will only deliver temporary remedy. This results in continuous short-term therapies and won't be serving as an ultimate cure, we talking about.
- **Side effects**: At any time, if foreign material is introduced into the body, they got attacked by the immune system of host body. Supposing we are about to insert a new gene molecule into brain of a patients, his/her immune system can attack this gene molecule, making the patient even more sick with several side effects. In other words, the patient may end up sensing sicker feeling after gene therapy than after taking regular antidepressant treatment.
- **Therapeutic targets**: As of now, numerous therapeutic targets for the specific use of gene therapy continued to be remain indistinguishable. While improving p11 level in the nucleus accumbens and in hippocampus look like a feasible option, more and research is essential to recognise its effect. Moreover, many patients have abnormalities with more than one genes that end up in depression. It is nearly impossible to treat patients with several genes simultaneously using gene therapy.

1.5 Therapeutic Targets of Gene Therapy for Depression

Last few years have seen astonishing developments in the field of genetics and neuronal science, along with new advancements in the molecular biology and nano science. The results from such studies allowed the researches to improve their knowledge about brain and its functioning. In the past decade, with the improved knowledge of brain functioning, various gene delivery approaches have been studied for the treatment of depression. Such gene delivery approaches include increase in the serotonin neurotransmission, enhancing serotonin sensitivity, increasing glucocorticoid susceptibility and increasing BDNF level in the brain.

Two genes which were extensively studied to increase the neurotransmission of 5-HT in brain includes SLC6A4, commonly known as SERT (serotonin transporter) and p11 gene. A polymorphism of SERT gene (5-HTTLPR) with a short (S) variation of 14 repeats and a long (L) variation of 16 repeats has been proven to influence the activity of SERT. SERT plays role in presynaptic re-uptake of serotonin. By reducing expression of this transporter it's possible to increase the level of serotonin in synaptic cleft.

Brain cells communicate with each other by secreting messengers, such as serotonin, which bind to receptors located on the surface of receiving cells. Serotonin selective reuptake inhibitors (SSRIs), medications commonly prescribed for anxiety and depression, compensate for reduction in serotonin signaling by boosting levels and binding of serotonin to receptors. Previous studies have suggested that serotonin receptors are essential in regulating moods and in mediating the effects of SSRIs, but given the complexity of the serotonin system, exactly how these receptors work remains a mystery.

Above described gene therapy is based on serotonin level in brain, the next hypothesis is based on high cortisol level, HPA axis dysregulation, and its effects on depression. Genes responsible for HPA axis regulation are: NR3C1, FKBP5, CRH1

GR function can be restored to its normal level which is mostly disturbed because of hyperactive HPA axis (hypothalamus-pituitary-adrenal), either by inducing number of receptors or by increasing GR dependent nuclear translocation and GR dependent gene transcription in depressed brain, it may possible to overcome depressive symptoms.It is postulated that Mice with an increased glucocorticoid receptor gene dosage show enhanced resistance to stress. In a study, the molecular events associated with the FK506 binding proteins (FKBP) -52 and -51 response to cortisol exposure in neuronal cell cultures and their effect on GR translocation was investigated. It was noted that FK506 altered nuclear localization of the GR and inhibited expression of GR-responsive genes.

Furthermore, siRNA knockdown of FKBP4 gene, coding for the immunophilin FKBP52, inhibited cortisol-activated GR nuclear translocation, while knockdown of FKBP5, coding for immunophilin FKBP51, was associated with increased baseline GR nuclear localization. Depression associated with HPA axis hyperactivity can also be regulated by CRH (corticotropin releasing hormone). A study of shCRH1 mediated gene silencing of CRH1 gene was performed and it exhibit phenotype characterized by reduced anxiety related behavior.Another hypothesis of depression depends on neuroplasticity mostly based on BDNF- which plays important role in the development of nervous system and TrkB dependent BDNF signaling pathway. In depressed patient, lower level of BDNF mRNA and that of TrkB was found. So gene therapy based to increase this gene expression levels can be applied.To explore how a particular serotonin receptor (5-HT1B) functions, Greengard and colleagues conducted tests to find out what proteins these receptors interact with in brain cells. They found that 5-HT1B interacts with p11, and according to Greengard, p11 plays a role in the recruitment of receptors to the cell surface where they are more functional. By using a virus to deliver an extra dose of the gene p11 to the adult mouse brain, the protein expressed by the gene is thought to bind to serotonin receptor molecules and ferry them to the cell surface, positioning them to receive serotonin's signals from neighboring cells. While the gene therapeutics for depression have not been studies in humans or they are not in clinical studies level, the primary studies in mice involving p11 gene showed its efficiency. Recent researches have evidenced that a particular protein involved in progression of depression in many patient is known as p11 or S100A10 which involved in changing the modulation and effectiveness of serotonin in brain.

Table 1. 1 Genes involved in depression

GENE	PROTEIN ENCODED	ROLE
SLC6A4	SERT	Increases serotonin neurotransmitter by decreasing presynaptic serotonin transports
S100A10	p11	Affects serotonin signal by fetch out serotonin receptor on cell surface
Fkbp5	FKBP51	Bound to GR, so corticoids cant bound to gr, affecting gr translocation and its trafficking
NR3C1	GR	Increases number of gr

CRHR1	CRHR1	Decreasing CRH level , important in negative feedback mechanism of glucocorticoid
BDNF	BDNF	Play important role in neuroplasticity
NTRK2	TrkB	Imp in BDNF signaling

1.6 Scientific research in last decade for depression

Last few years have seen astonishing developments in the field of genetics and neuronal science, along with new advancements in the molecular biology and nano science. The results from such studies allowed the researches to improve their knowledge about brain and its functioning. The mystery surrounding depression is yet to be solved but at the same time these progresses have moved us very close to the solution. There are many proofs that suggest the role of genetic abnormalities play a huge part in the progression of depression. While the gene therapeutics for depression have not been studies in humans or they are not in clinical studies level, the primary studies in mice involving p11 gene showed its efficiency (14). Recent researches have evidenced that a particular protein involved in progression of depression in many patient is known as p11 or S100A10 which involved in changing the modulation and effectiveness of serotonin in brain (15).

2015: It is a fact that depression is not only a problem for those suffering with it but also has a significant effect on the economy. Right now, there is no measurable quality for the assessment of an individual is depressed or not. In most of the cases, psychiatrists prescribe medication based on their interaction with person which can vary one therapist to another for the same patient (16). This may result in insufficient relief from mental illness for some patients. Auspiciously with the advancement in science, it is already been proved how individual gene and protein or other biomarkers may play role in depression. For the treatment consistency companies like GeneSight endeavour in the field of antidepressant treatment response and some blood tests to measure biomarkers are being under investigation (17).

An article published in 2015 has discussed in which way genes can affect depression and how protein/RNA biomarkers derived from RBC may help identifying depression (18). The article also focused on using entire genome (human) and revealing individual genetic foundations accountable for depression. If we can identify all the deformities in proteins responsible for depression and genes responsible for it, the pharma company would be able to provide new formulations that can have new targets for successful depression therapy (19).

2013: The most important evidence for the futuristic development of depression treatment was found in 2013, a protein known as p11 (S100A10) was found to play a very vital role in depression pathology (20). This protein has efficiency to change signalling potency of serotonin. In one study in same year, researchers have found the level of p11 protein in control, amidst the patients receiving antidepressant treatment. Furthermore, it was also documented that p11 protein also decides whether the patient respond to antidepressant treatment or not. Precisely, patient's vulnerability to depression may be linked with p11 protein and serotonin. This p11 protein act together with serotonin receptors and help anti-depressants to elevate the mood (21). Researchers worked on peripheral treatment approach for depression by working on "serotonin 4" receptors found most prominently in gut. The research on p11 protein lead to this approach as p11 found to affect depression treatment by vital interaction with these "serotonin 4" receptors (22). In such scenario, it is possible in future to target "serotonin 4" receptors without need of crossing BBB to provide rapid onset of action. Work in this area has considered the role of p11 gene therapy for the possible treatment based on these findings. By individual treatment of p11 gene, patient may also not require the present antidepressants to achieve better mental piece or freedom from depression.

In the same year, some researchers have studied the role of gene for efficient innovative treatment of psychiatric disorders that also includes major depression (23). It is recognised that the person with mental dysfunctionalities usual have abnormal problems with brain region.As a result of abnormal functioning of brain, researchers ventures the gene therapy may fix the problem and in process deliver the considerable assistance to patients. They found that for the effective gene therapy identical procedures is a requisite for it to be effective as was used for brain stimulation (24). It was assumed that for such simulation for each and every time neurosurgeons is required. Even though all these problems associated with gene therapy, it is a futuristic approach to treat various CNS disorders.

2012: Many studies have proved that present antidepressant therapy is not beneficial to everyone (25). A molecule that is efficient for one patient may not be as effective in another patient. The reason behind failure of treatment is the involvement of different types of gene which may exhibit similar kind of symptoms in individual patients (26). As per one finding in 2012, the specific gene as well as their interaction with environmental factors play part in depression progression (27). They also suggested the involvement of genes in predicting survival of an individual with the help of antidepressants. Researcher

concluded that by investigating the difference between various genes involved in depression, it's possible to eradicate root cause of depression.

2011: The field of psychiatry has never believed in the concept of individualisation for different diseases that is the main concern. For instance, a few patients may be diagnosed with the same disease depression but root cause behind both of them may be varied; one patient may be depressed because of low serotonin level while other may be on account of low norepinephrine or dopamine level. In spite of these characteristics, there is no single way to understand the cause of depression in individual patient, there is no single way to understand the cause of depression in individual patient (28).

Psychiatrists are not only failed in measuring level of neurotransmitters in patients suffering from depression but also the reasons behind imbalance of these neurotransmitters are unknown to them. Moreover, in some patients depression might not be resulted from neurotransmitter problems; it could be any reason other than that. A report published in the same year recommended to understand how difference in genes and environmental factors affect the depression (29).

Although patients suffering from depression would be ready to try out new therapy as gene therapeutics but it's far from reality. One such a futuristic target is p11 gene which produces a protein that particularly binds to serotonin receptors present in brain. p11 at below par quantity in nucleus accumbens of animals result in depression (30). While upsurging the level of p11 protein in nucleus accumbens diminishes the depression level in animal model. As a result gene therapy involving p11 gene is ventured as a promising approach for humans (31).

In spite of venturing it as a promising approach in humans, many hurdles need to be solved before actually trying it in human. More research in animals is essential to determine its long term side effects knowledge of which is still in early stages. Adding to this problem, numerous regulatory requirements are to be fulfilled before it can be used in humans specifically for depression (32).

2010: The main principle behind depression is the problem in serotonin signalling property and one of the reason behind serotonin functioning is level of p11 protein (33). It has already been ascertained that p11 works as a protein who binds to 5-HT1B receptors as well as 5-HT4 receptors (34). Scientists have already found that mice and rats with p11 knockout gene are extremely susceptible to depression which results in hypothesis that p11 actually plays significant role in changing mood. Moreover, they have also found the nucleus accumbens and hypothalamus as a main area to work for p11 protein (35). When

siRNA for p11 mRNA with adeno-associated virus was administered in mice they were specifically able to inhibit the level of p11 in nucleus accumbens. It was the specific inhibition of p11 protein in nucleus accumbens resulted in depression. (36) After this work, scientists emphasised on the fact that increase in p11 level specifically with gene delivery may be beneficial to the ones suffering with depression.

2010: Alternative research also noted the fact that p11 level in nucleus accumbens mat able to change the depressive behaviour in animals. Those animals expressing higher level of p11 protein showed normal behaviour with less depressive tendencies (37). Same way, animal with reduced p11 level or p11 knockout mice likely to demonstrate depression in their behaviour (38). They also investigated the level of p11 protein in 17 depressive patients and compared them with another 17 normal human without any behavioural impairment. And as predicted, the patients with mood disorders, were found with drastically lower level of p11 protein level than those of normal human beings (39). These findings recommend to increase the brain protein level of p11 in the patients suffering from depression symptoms. It was supposed that the upsurge in p11 protein level in depressive patients might result in normal behaviour. But it was not known that the increase in p11 level after certain point would affect the unpleasant feeling experience or not (40).

Moreover, in the field of genetics for depression, p11 is just one of the promising targets to effectively treat depression.It's effectively transport 5-Ht receptors particularly, 5-HT1b receptors to the cell surface and enhanced the chances of communication between serotonin and 5-HT receptors (41). Directing the future research towards improvement of p11 protein may also be useful for the patients with resistant depressive disorders.

2009: In one published article in 2009, scientists have proposed the various complications in current treatment regimen of depression. As a result, it is imperative to emphasis on the probable target involved in the depression. They suggested to examine phosphodiesterases (PDEs) which plays role in metabolism of cyclic AMP and cyclic GMP (42). Examination in both animals and human proposes that PDEs play part in depressive behaviourand anxiety. Moreover, many genes influencing PDEs has been found out to affect depression and schizophrenia (43).

Lately, it was observed that the inhibition of PDE4B resulted in cognitive enhancement or super intelligence in mice along with powerful anxiolytic effects. But there was effect on mood was found after inhibiting PDE4B, therefore it was proved that PDE other than PDE4B might play the role in depressive behaviour (44). Hypothesing genes affecting

PDE (or PDE knockout mice) linked with depression can be discovered, it can be used as a potential target for depression.

2008: A report submitted in 2008 demonstrated almost half of the depression patients and quarter of patients suffered with anxiety problems do not get any relief with present antidepressants. These problems can be solved by the principle of individual medicine practice e.g. one patient may get benefit form one particular medicine because that particular medicine act on the specific neurotransmitter deficit or gene defect in that patient (45). Another patient B may be suffered with same symptoms but with varied underlying cause, in that case person B would not be responsive to the therapy person A receiving.

Scientists have worked upon many genes affected by present antidepressants and also on many genes responsible for the progression of depression. In large studies of patients suffering from depression, polymorphism in serotonin transporting gene (SERT), was found which included 5-HTTLPR and STin2 (46). Additionally, many more genes involved in depression and those affected by antidepressants were found which comprised of specific 5-HT receptor genes, BDNF, P-glycoprotein, G-proteins, TPH1, TPH2, MAOA, NET (norepinephrine transporter), FKBP5, and CYP450 (47). For instance, it was agreed upon that CYP450 is actively participate in metabolism of antidepressant drugs, which certainly influence the effects of many antidepressant drugs and their strength (48). Scientists are certain about using particular gene to forecast effects of antidepressants in individual patent in future.

2007: Researchers have noticed that patients on antidepressants are on contradictorily increased the risk of depression and suicidal tendency. The medicines that is said to be altering neurotransmitters level might not be affecting them at all in certain patients may be the reason behind that. Variation in genes in individual patients is responsible for such effects which may result in deteriorating effects in patient behaviour. Scientists were still not sure what ultimately leading to the suicidal tendency while patients on antidepressant treatment (49). Therefore, they concluded to work upon protein and genetic biomarkers for drugs induced suicidal tendencies. They collected DNA samples from around 2000 patients to carry out Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study. (50) These samples were evaluated for around 770 single nucleotide polymorphisms (SNPs) amid 68 genes.These variations in genetic infrequencies were evaluated in 120 patients who experienced antidepressant induced suicidal tendency. They found that genetic biomarkers- GRIA3 and GRIK- both of them were associated with the

problem aforementioned. Researchers have reckoned if more and more studies are devoted in this direction, it may be possible in near future to analyse suicidal problems in individual patients suffering from this antidepressant curse (50).

2006: A publication in 2006 stated the susceptibility of PDE genes to play part in progression of depression. Scientists worked upon if particular gene or protein biomarker of PDE in fact affect the chances of depression in patients (51). Researches collected DNA samples from 300 patients and they were compared with 350 normal (non-depressed) individuals of Mexican and Americans origin). Aggregate of 22 genes this PDE family were evaluated to conclude whether any distinguishable dissimilarities present between two class or not (52).

Polymorphism in PDE9A and PDE11A showed the positive results in studies that increased the probability of depression.Those individuals benefited from antidepressant therapy found to have polymorphism in PDE1A and PDE11A genes. Researches also established that GAACC, a halpotype of PDE11A is linked with depression and anti-depression treatment (53).

1.7 Vectors for the gene delivery

At present, the major problem associated with practical use of gene therapy is the development of delivery system which is efficient as well as non-toxic in nature. Gene expression is possible only when the gene therapeutic is transported to the cells and presently it is probable to achieve local temporary gene expression when naked DNA is inserted in muscle tissues (54). It is tough to acquire systemic effect with a single administration of gene therapeutic, which in most of the cases result into lower level of gene transfection in most of the major organs. There are numerous explanations for it:

- Naked DNA cannot transfect itself in target organ *in vivo*;
- When injected systemically any form of oligonucleotides is degraded by serum nucleases;
- Influx of DNA plasmid into its effector site i.e. nucleus is hindered by many barrier present in cellular compartments.

Vehicles to transfer genetic material effectively into target cells can be divided in viral and non-viral vectors (55). For the last few years, many efforts have been made to construct a vector which is not originated from virus and results in discovery of non-viral vectors allowing a superior flexibility in the capacity of DNA transportation, bypassing the immunity of host body and increase the safety (56). Several lipids, peptides, protein and

polymer based neon systems are presently in the stage of development for the efficient gene delivery. Typically complex should carry the net positive charge value that allow the DNA-vector complex to interact with the cell membrane containing negative charge and results into internalisation by the cell which occur through any process of endocytosis (57).

1.8 Non-viral vectors for gene therapy

Gene therapy is a capably strategy for rectification of pathological disease or other chronic condition with the help of genetic modification. In the groundwork, gene therapy comprises of deliberate modification of protein expression through gene expression using DNA, mRNA, miRNA, and RNAi molecules, such as siRNA or small hairpin RNAs (shRNA), and antisense oligonucleotides (AONs) (58). Because of its bigger size and higher negative charge, such macro biological molecules need some kind of career for their delivery. In last two decades, many studies for gene delivery were initiated not more than 5-6 products (Gendicine, Oncorine, Rexin G, Neovasculgen, and Glybera) are in global market and none of them have approval of USFDA (59). Most of the experiments were failed to reach phase III demonstrating potential challenges with such therapy. Among the viral vectors, 90 % of vectors are comprised of adenoviruses, adeno-associated viruses (AAVs), lentiviruses, or retroviruses. These viral vectors have considerably advanced the gene delivery technology but they have many pitfalls including very less DNA integrating capacity, carcinogenic, trigger immune response (60). These shortcomings lead to the nonviral delivery careers with the capacity to solve many of these issues owing to recent advances in material science, improved knowledge of structure and chemistry of nucleic acid as well as nanobiotechnology.

1.9 Cationic liposomes for gene delivery

When oligonucleotides like double stranded DNA (dsDNA) or siRNA are complexed with cationic liposomes, the formed complex is known as “lipoplex”. So formed lipoplex or polyplex (complex between oligonucleotide and cationic polmer) can be used as a nonviral vector for gene delivery (61). Nonviral vector system is not as strong as viral vectors in their gene transfection efficiency but they are not expensive, non-immunogenic and no limitation in packaging of DNA with vector. Moreover, the cationic liposomes are not as toxic as their non-viral counterpart as they are formulated from phospholipids (biological lipids). The positive charge present on the surface of cationic liposomes interact with anionic nucleic acid and eventually formed net positive charge of complex

assists in contacting negatively charged cell membranes (62). Cationic polymers are diverged in nature from cationic lipids in their hydrophobic parts and they are water soluble. Cationic lipids can also be modified in variety of geometry, shape or length. Functional groups can also be added to the cationic lipids very easily.

Cationic liposomes are the utmost promising non-viral vector system with polycationic naturefor gene delivery. Cationic liposomes are most frequently formed from cationic lipid DOTAP, neutral lipids (DOPE and HSPC) and cholesterol. Their main advantages are their uniqueness to incorporate both water soluble and water-insoluble drugs, non-toxic nature and targeted delivery to the site of action whether it is cancer cells or brain cells (63). The concern with liposomal system is their fast elimination from body with Reticulum Endothelium System (RES) and their ineffectiveness in achieving continuous and sustained delivery for longer period. This problem can be solved with PEG coating over liposomal surface. Cholesterol or DOPE (helper lipids) are usually uses in such systems to enable lipid exchange with improved membrane destabilising property which helps nonviral vector containing gene to escape endosome (64). In the same way, HSPC (phosphatidylcholines) are used as a part of liposomal system which provide stability to vector by creating stable bilayer which ultimately helps gene to reach its site of action.As a result, lipoplexes formed form phosphatidylcholines as their integral part help gene in reaching the site of action without any degradation (65).The effectiveness of liposomal system to deliver gene, rest on the geometry, shape and charge ratio between liposomes and DNA,type of helper lipid and the site of action.

Lipofectamine is a marketed liposomes with net positive charge having a high transfection efficiency for nucleic acids.It has ability to complex and can carry negatively charged DNA. This positive charge on liposomes help them to put up a fight with electrostatic repulsion of cell and can be easily engulfed by the cells. In addition to nucleic acids, this Lipofectamine is also helpful in delivery of siRNA to the site of action (66). In one recent paper Rasoulianboroujeni et al. (2017) established the efficiency of cationic liposomes for transfection and expression of LacZ-gene comparable to Lipofectamine 2000. They used liposomal formulation with formula of DOTAP/DOPE/cholesterol in a molar ratio of 1:1.5:2 formed using thin film hydration method which was further lyophilised for stability purpose (67).

1.10 Basic principles of cationic lipid-mediated gene transfection

The primary step in formation of lipoplex is the condensation of large sized DNA molecules with positively charged liposomes by electrostatic interaction between positively charged lipid present in cationic liposomes and negatively charged DNA. This interaction results into impulsive self-assembly into nano sized particle known as "lipoplex" (68). This assembly helps in shielding DNA from nucleases present in blood or extracellular medium. Using excess of positively charged lipid, helps the lipoplex surface in gaining net positive charge which subsequently helps in intracellular delivery of complexed DNA with the interaction of heparin sulphates and other components present on cell surface. This interaction results in endocytosis but still cellular uptake as a result of lipid-cell fusion cannot be left out. The complexed DNA should be able to escape degradation in acidic pH of endosome to get released into cytoplasm.The DNA released into cytoplasm compartment should next be proceeded with entry into nucleus to facilitate gene expression. Now in the nucleus, the DNA should be able to detach itself from positively charged vector and the work involving microinjection has confirmed that the DNA remainders in form of condensed product cannot transform itself (69).

The efficacy of lipoplex to transfect gene can be studied in many ways. One of the way is to calculate the percentage of cells transfected or the amount of transgene protein produced as a result of transfection. The another way is to evaluate their efficiency using cell lines but this efficiency depends on the cell line chosen to work with, as a result this type of experiments are no considered dependable (70). This reflects the important role of how the relative efficiency of different cationic lipids used for the experiments and highlights that the quantifying of the different cationic lipids should be analysed using appropriate method as per their proposed use.

Felgner et al first introduced the cationic lipids in their attempts to transfer DNA encapsulated in liposomes. DOTMA (N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride) was the first used lipid with a quaternary ammonium group linked to two unsaturated non-aromatic carbon chains using ether groups (71). With the success of DOTMA, soon the DOGS (dioctadecylamido-glycylspermine) was synthesised and the efficacy of the formulated vector DC-Chol (3b-[N-(N', N'-dimethylaminoethyl) carbamoyl) cholesterol) was evaluated, as the name itself denoted, cholesterol was an integral part which formed hydrophobic section. It should be noted that the transfection ability of cationic lipids which usually are no able to form stable bilayer can be improved with the use of colipid DOPE (dioleoyl phosphatidylethanolamine).

DOPE inclusion thought to increase the endosomal escape capacity of liposomal formulation intracellular owing to its fusogenic properties playing a significant role in membrane disruption of endosomes. The use of these initial helper lipids showed the effects on transfection efficiency of lipoplex which was followed by a very thought provoking stage as there was no clear indications between the vector synthesis and its transfection efficiency (72). This partial understanding led to very complex steps for the measurement of transfection efficiency. Therefore, the synthesis of modified or totally lipids is necessary and they are not for the sake of addition to already formulated liposomes but have an important role to play in gene delivery. As a result, many novel conceptualised lipids were synthesised and their transfection efficiency was measured however their mechanism of transfection was the main area to look after. The mechanism for endosomal escape of liposomes containing cDNA is shown in figure 1.3.

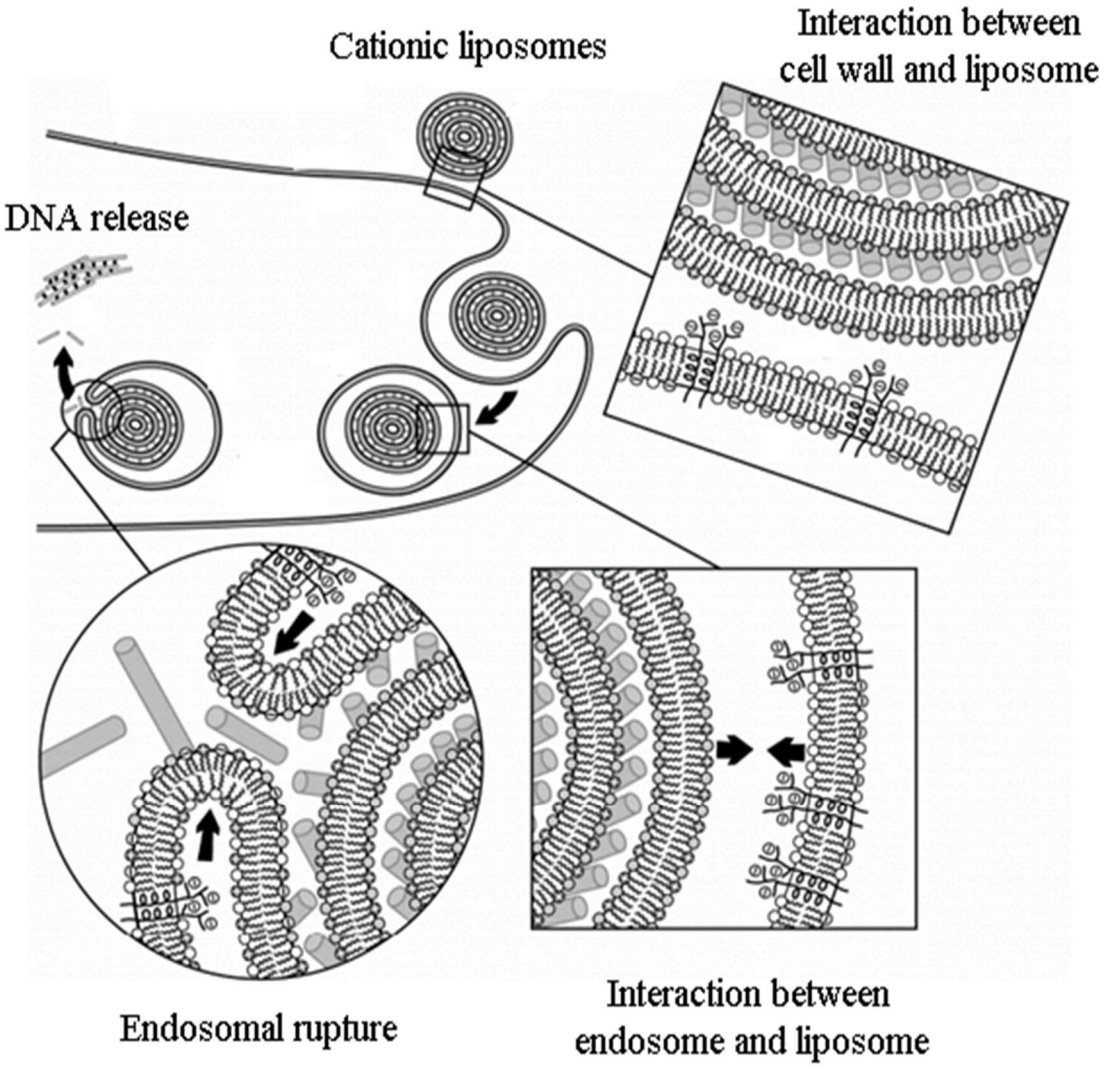

Figure 1. 3 Endosomal escape mechanism for liposomes containing cDNA

1.11 Formation of cationic lipoplexes

1.11.1 Driving force and DNA condensation during lipoplex formation

Even though the transfection efficiency of various novel synthesised cationic lipids has been increased in comparison to non-viral system, the mechanism for the formation of lipoplex is yet to be fully understood. Felgner et al. established the electrostatic interaction between positively charged lipid and negatively charged nucleic acid as the reason for the formation of lipoplexes (73). Kreiss et al. reported that the interaction between repulsive forces and elasticity forces between the hydrophobic moiety of lipids define the morphology of lipoplex (74). A single molecule of DNA is bounded by adequate cationic lipids to fully nullify the anionic charge of DNA and deliver a final complex system with a net positive charge that can attach itself to negatively charged cell surface that is the ultimate criteria for deciding transfection (75). For the understanding of lipoplex and cell interactions, it is significant to recognise the thermodynamics the formation of lipoplex. Lipoplex formation is actually a very vigorous event, comprising of unrestrained interactions between cationic lipids and DNA. Pozharski et al. suggested that the formation of lipoplex is an endothermic reaction which involves absorption of 1 kcal of energy per mole of lipid or per mole of energy per unit DNA charge moreover, lipoplex formation was determined by very high amount of entropy linked with the firmly bound counter ions released from DNA surface and lipid vesicles (76).

Two developments simultaneously take place during the lipoplex formation a) membrane fusion by DNA and b) DNA folding by liposomes. DNA folding by liposomes is a prime event and bunches of cationic molecules (lipids) attached themselves to DNA and small rod type structure in which negatively charged folded form of DNA is completely encapsulated within liposomal vesicles. A concentric configuration arises from positively charged liposome and anionic DNA complexation as documented by Scarzello et al (77). DNA molecules experience an intense condensation to a tightly packed, usually well-arranged toroidal shape known as ψ- DNA, a distinct phase that is structured like liquid crystals of crystals, which has a chirality of left hand owing to both the alteration and condensation of positively charged lipids. At the time of lipoplex formation, lipid and DNA both experience alteration into dense quasispherical compact of around 200 nm size and they are able to form complexes with well-organised lamellar structure.

1.11.2 Lipoplex structures

Basically two prototypes of lipoplex formation model was employed one of which is based on DNA absorption on the surface of liposomes i.e. an external model and the

model is external model which stated the DNA coating by lipid bilayers. In a model for lipoplex formation, as proved by Felgner et al nucleic acid or DNA with negative charge electrostatically complexed to cationic liposomes (78). When lipoplex formulated from multivalent DDAB or DOTAP observed under cryo-TEM, DNA was seen aggregated in multilamellar vesicular structure even at very low lipid-DNA ratio i.e. at very low level of lipid. When surplus amount of positively charged surfactant was used in comparison to charge of DNA, primes it towards the DNA entrapment between the lamellas of multilamellar liposomal vesicles, even liberated form the choice of surfactant. In a mechanism involving three steps, numerous bean structured complexes aggregate themselves into analogous or upright orientation, at last whose diameter is nearly equivalent to particular plasmid DNA i.e. around 200 nm, inferring the very small effect of condensation. Ellipsoid shaped particles were formed when the lipoplex were formulated from SAINT-2, a pyridinium based lipids which complex themselves very easily with DNA. When this SAINT-2 lipid was mixed with DOPE in equimolar ratios were incubated with DNA, ellipsoid shaped liposomes were converted into round shaped vesicles. Latest research confirmed that during the complexation, condensation and transportation stage, lipoplex was in lamellar shape whereas hexagonal form was seen upon the contact of lipoplex with cel surface. Further, during their engulfing or endocytosis stage, a well-ordered tubular shape was visible and accumulate themselves into endosomes. The observation showed that the well-ordered cationic lipoplex would be in multilamellar shape LC α and in the same way, hexagonal structures showed inverted hexagonal HC II and HI shape. Studies suggested that the most of the lipoplex formulation confers lamellar type structure with plasmid DNA aggregated between the positively charged lipids. A finding concluded that in between LC α and HC II shape one momentarily spaghetti type of structure is existed which probably assisted as a sign to HC II phase. Even though the formation of liposomes seem to be very basic and simple, it has the potential to determine lamellar level, shape and transfection efficiency of lipoplex (79). When two step lipoplex formation mechanism is involved, the first step is accredited to electrostatic binding of DNA to liposome which is followed by restructure, rearrangement, phase changes and fusion of vesicles. Various shape of liposomal structure is shown in figure 1.4.

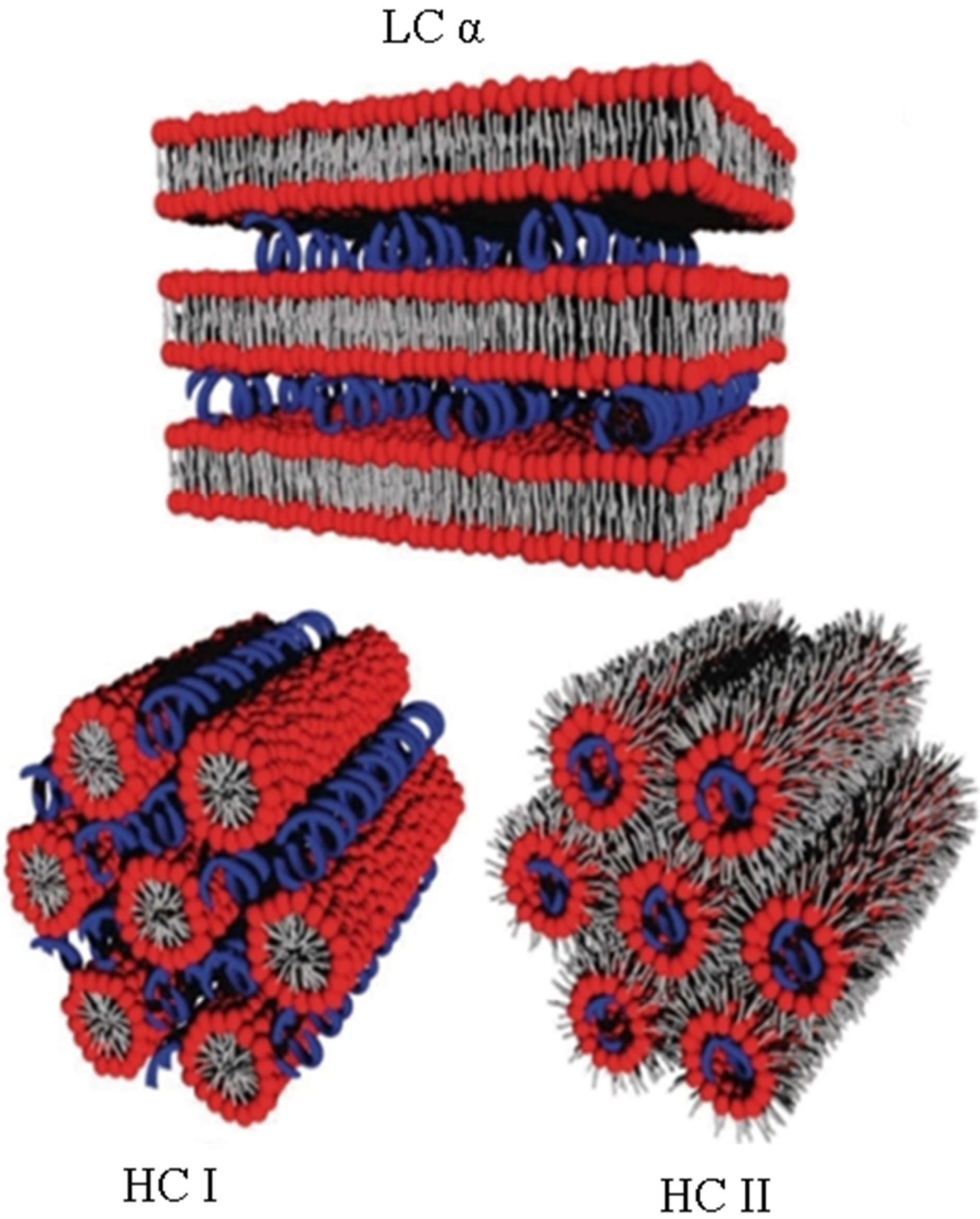

Figure 1. 4 Various shape of liposomal structure

1.11.3 Factors affecting phase behaviour

It is already identified that the stable lipoplex formation hinges on many parameters like characteristic of cationic lipid and helper lipid, charge ratio of liposomes to DNA. A few thermodynamic characteristics of lipoplex structure allow them to confer only two primary shapes i.e. LC α and HC II which was already been discussed in previous section (80). Now the characteristics that lead to the favoured spatial structure will be discussed in next sections.

1.11.4 Characteristics of cationic lipid

The association of phase behaviour of positively charged lipids and final structure after DNA condensation with them is particularly important for the estimation of their transfection efficiency. Theory of curvature has stated that the molecule shape which helps in determining the membrane curvature, Co = 1/Ro, also helps in determining the curvature of lipid vesicle according to same equation i.e. C=1/R where "R" represents the actual radius and "Ro" represents the natural radius curvature of liposomal vesicle monolayer.The actual curvature Ro represents lipoplex structure e.g. when C=O the favourable structure would be LC α (lamellar structure), inverted hexagonal (HC II) structure would be preferred structure and dominant structure would be hexagonal HI (CoN0). During the HI phase, lipid micelles of tubular shape are organised on a hexagonal shaped matrix and rode shaped DNA which is relied on the degree of hydration, are organised on a honeycomb matrix in the space between lipid micelle. Leftover place in HI structure is occupied by water, ions and headgroups. Sunfish et al has established that divergent in their hydrophobic part, the dependent tendency of lipoplex structure on the hydrophobic region size could be articulate by P=V/a·l; a packing parameter with no dimensions where "V" represents the chain volume of hydrophobic part, "a" denotes the ideal area of headgroup and "l" represents the hydrophobic tail length. Lipids with four carbon chains shows higher tendency to persuade HC II phase owing to their huge cross section area (81). If we restrain amphiphiles to pyridinium group, the lipoplex have a tendency to adopt clear cut HC II phase structure with the increase in carbon numbers in alkyl chain. It is also well-recognised that introcduction of unsaturation in alkyl chain leads the lipoplex towards adopting HC II type of structure which may be because of improved cross area of hydrocarbon chain but distinct positions of unsaturation in alkyl chain do not infer with any changes in morphology.

1.11.5 Ionic strength and temperature

Lipoplex in LC α phase is the most stable phase in presence of various ions and varied range of temperature. On increasing the temperature, LC α would converted to HC II phase which eventually affect the mobility of lipid chains. On increasing the temperature, the outcome is decrease in "a" as a result of hydration shell reduction and growth in alkyl chain volume "V" because of its increased mobility. On increasing the concentration of ions, lipid mixture aggregates into a comparatively stable cubic phase which in the end, experience a transition into HC II phase after complexation with DNA (82). Reason behind this change is that on increasing the concentration of ions (e.g. Sodium Chloride), the key moment is the repulsion between head group of lipids which primes it towards decrease in head group area "a" and ultimately, intensification of parameter for packing "p", constant with the change into HC II phase.Lately, with the use of Nile Red assay, Wasungu et al. proved that lipoplex synthesised from gemini surfactants synthesised from natural sugar, GS1 and GS2 experienced a non-inverted micellar phase conversion from lamellar phase on lowering the pH from 7.0 to 4.5 (neutral to mild acidic pH), resulting in contrary results from the lipoplex formulated from SAINT-2/DOPE, a traditional HC II forming system (83). Thus, these two liposome systems, GS1 and GS2 formed a stable system even in presence of salt and pH changes because of its lamellar orientation gave rise to many surfactants and lipid modifications that are stable in physiological condition.

1.11.6 Helper lipid

In the presence of helper lipids such as DOPE, the regular packing parameter "P" increases and lead towards a mixed bilayer system. Latest researches have shown that lipoplexes formed from helper lipids would encourage HC II organization. A conversion from LC α phase to the HC II phase possible be predicted by increasing the mole fraction of helper lipid DOPE in formulation, in a way to control the curvature radius "Ro" of vesicles formed from lipid bilayer (84).On another side, the capability of DOPE to regulate of gain the control over structure of lipoplexes form from cationic lipid is also governed by shape of the particular lipid which is dependent on the headgroup region to alkali chain area proportion. On the contrary, it was also expected that lipids like dioleoylphosphatidylcholine (DOPC) would restrict the capacity of cationic lipids to encourage structure that is not bilayer in nature (85). In the case of anionic lipoplex, to pretend interaction between lipoplex and endosomal membrane, SAINT-2/DOPE lipoplex exhibited an impeccable hexagonal structure on the other hand SAINT-2 along with DPPE lipoplex showed intermediate of lamellar–hexagonal structure where as it should be

showing lamellar phase owing to the presence of dipalmitoylphosphatidylethanolamine (DPPE).

1.11.7 DNA concentration (or lipid to DNA ratio or L/P ratio)

Globular complexes are formed mostly at higher charge ratio of lipid/DNA.DNA forces larger acyl chain structure to emerge and they are complexed by an imperceptible thread exposed by globules in Brownian motion state. Lipoplexes of larger size and multlamellar in structure, have broad chances of mixing together with DNA at a large range (+/-) ratios as exposed by sedimentation in sucrose solution of different density gradients (86). Precondensation of DNA with one chained cationic surfactant CTAB and subsequently complexation with DMPC: DOPE (1:1) liposomes, resulted in ternary complex having multilamellar structure proved by cryo-TEM images. The research work on complexes formed by DNA with CTAB, with the help of cosurfectant hexanol and analysis with small angle XRD, after increasing the amount of hexanol, lipoplex confirmation changed from, hexagonal shape (HI) to lamellar structure (LC α) which is ultimately converted into inverted hexagonal (HC II) confirmation, particularly at low DNA concentration (87). Now at higher hexanol and higher DNA level, inverted hexagonal to lamellar confirmation was observed. Thus, lamellar to inverted hexagonal to again lamellar conformation was obtained as the concentration of DNA was increased in presence of higher level of hexanol. Another result revealed that at varied positive liposomes to negative DNA charge ratio, the formation of cationic lipoplex was not affected above the isoelectric point, the point at which the charge on DNA and cationic lipids are almost equivalent.

1.11.8 Zeta potential

The most important parameter that can affect the interaction of cationic liposomal surface to anionic lipids is the zeta potential of the liposomal vesicles. Usually zeta potential is actually important for determining lipid to DNA ratio and the morphology, shape and structure of negatively charged lipoplex is rather different than that of positively charged lipoplexes. Lipoplexes with positive charge on their surface resemble the multilamellar structure in their aggregated form (LC α). Negative zeta potential concludes the free form of plasmids or extended DNA string like molecules. It is likelihood that at lower ratio of lipid to DNA, “external” structure of lipoplex is formed in which almost all the DNA molecules attached themselves to the outer surface of complex rather than to be encapsulated in vesicle (88). However, zeta potential is one of the important parameters affecting transfection of DNA but it is not the only parameter affecting transfection, other than transfection efficiency factors including lipoplex structure, morphology, process and

formulation parameters including incubation time. Although aforementioned parameters can never be neglected but the shape of cationiclipid and helper lipid (e.g. DOPE and DPPE), lipid to DNA charge ratio are the key factors leading to the formation of lipoplex structure and morphology. The most important principle we can pick up from above mentioned theories is that if the researchers can relate the efficacy of DNA transfection with the lipoplex structure and morphology, it is possible to assume lipofection (ability to transfect gene using liposomes) based on factors leading to appropriate lipoplex morphologies. (89)

1.12 Delivery of DNA to the central nervous system

1.12.1 Targeting lipoplex across BBB using systemic delivery

The molecules exchange between blood circulation and brain parenchyma is controlled by the following three barriers which include blood brain barrier (glial cells plus endothelial cells found in blood vessels of brain); epithelium of choroid plexus which actually is sandwiched between blood and CSF (cerebrospinal flood) and epithelium of arachnoid which differentiate blood from subarachnoid cerebrospinal fluid. The endothelial cell layer forms the BBB, this layer establishes capillary which is trailed by a membrane containing pericytes as well as astrocytes. These cells which are the part of pericytes are used for various functions including clotting of blood, venous regulation, generation of neurons, activation of blood cells etc. this barrier supports in many functions but their main part is to protect brain from foreign external materials (90). This protection from foreign material is attained because of the presence of capillary bed in brain which is differ from capillaries found in peripheral system in the below listed features:

- Tight junctions help in reducing intracellular space between nearby capillaries;
- The transfer of materials by the process of pinocytosis is reduced considerably;
- Leaks between the areas of intracellular space is almost lacking.

In BBB, tight junctions present in the area of endothelial cells helps in maintaining the extraordinary electrical resistance which is around 1500–2000 $\Omega.cm^2$whereas resistance of 3.33 $\Omega.cm^2$ is found all over body. A wide range of molecules across the brain is transported through a high concentration of P-gp also known as P-glycoprotein, one of the most promoted protein for transportation which is a part of ABC (ATP-binding cassette), proteins associated with multidrug resistance and resistance protein for breast cancer. The above mentioned proteins help to carry out molecules from brain to other body tissues using efflux pump (91). On the contrary, there are certain proteins which help in

transporting molecules from other body parts to the brain known as influx transporters which are the members of OATPs (organic anion transporting polypeptide family) and OATs (organic anion transporters), which not only helps in influx but also facilitates efflux process.

Most of the substrates across the brain is transported through the transcellular route because of tight junctions present in endothelial cell layer. Simple diffusion helps only when the small lipophilic molecules of size lesser than 400 Da is involved. In short BBB, is not a passive membrane made up of lipid layers, but in fact it is an interface which is dynamic in nature having both the transporter components, physical and metabolic transporters. Various endogenous transportation systems are found at the surface of BBB and are liable for transporting essential and hydrophilic substrates, some of them may be macromolecules. Essential molecules which are hydrophilic in nature like proteins are transported through the process of endocytosis which can be receptor specific transcytosis or less specific adsorption based transcytosis. Ligands present on the surface of endothelial cells assist in transportation of large molecules like **insulin** and transferrin. In a way we can say that the receptors present on the surface of brain and fund most abundantly are **insulin receptors** and transferrin receptors. For the transportation of proteins like albumin, adsorptive endocytosis prove to be an efficient mechanism which is attributed to the electrostatic interaction of cationic proteins to negatively charged cell surface of BBB (92).

Monoclonal antibodies (mAbs)

For the targeting of drugs, proteins, peptides or any nano career, monoclonal antibodies are on of the most prominent options. Main hurdle in using them as a targeting moiety is its production which was solved by Köhler and Milstein who established a technique fondly knows as hybridoma technology that permits the production of large quantities of monoclonal antibodies which is very specific to its proteins. This easy production of mABs lead to use of antibody itself or as a part of immunoconjugates as a part of therapy. Antibodies are huge molecules around 150 kDa, which is hydrophilic in nature with long half-life in serum. The large sized molecules face problems in entering BBB with the help of simple diffusion. Experiments proved that the antibodies injected systemically have very lower tendency to be localised in brain or low efficacy in crossing BBB. This lower tendency of crossing the BBB mixed with powerful apparent system results in very low accumulation of antibodies in brain. However, even though their lower tendency in

crossing BBB they can be used to transport drug molecules or nano carrier containing any kind of therapeutic molecules though receptor mediated transcytosis (RMT). This RMT transportation mechanism appears to be one of the most encouraging approaches to transport therapeutic molecule across brain owing to their high capacity to incorporate molecules, lesser side effects and avoidance of efflux system. Insulin, insulin like growth factor (I & II), transferrin, melanotransferrin like high molecular weight and big molecules have receptors at the luminal side of BBb endothelial cells and helo via RMT to transport them. These transport system is saturbale in nature i.e. they can bind to ligands in finite quantity. Therefore, rate of diffusion through RMT is usually inferior to the expected transportation rate. The transferrin receptor (TfR) and insulin like growth factor receptor (IGFR) are two receptors found in brain at very high density in comparison to other organs, making the brain a suitable candidate for targeted delivery using these receptors. Despite their high density in BBB, their use for transporting therapeutic molecules is not much explored. High level of endogenous transferrin and IGF affect the saturation level of their respective receptors on BBB (93). For this reason, the antibodies against these specific receptor is preferable in comparison to their protein part.

The mAbs for Tfr and IGF do not contest with natural IGF and Tfr found in our body. From the two antibodies mentioned above, most of the work done against the Tfr or OX26 mAb. Immunohistochemistry and radiolabelling techniques have been extensively used for the study of targeting efficiency of OX26 mAb. The results of the studies showed that the OX26 can transport through BCEC (brain capillary endothelial cells) but not able to be accumulated in brain at considerable quantity. OX26 is used against the rat transferrin receptors, other monoclonal antibodies were also used to target the same receptor but in mouse which includes 8D3 and the RI7217 mAbs which also have association with human transferrin receptors *in vitro*, mostly due to its 86 % similarity with mouse receptors. In one of the *in vitro* work carried out by van Rooy et. al indicated that RI7217 attaches itself to the endothelium cells of human (94). They also found that the insulin receptor is one of the most profoundly established receptor of

Human brain endothelium cells.In one nother study by Kuo et al., 83-14 mAb was used to target the insulin receptors found on brain and it has confirmed the 10 times more efficeicny of same in comparison to mAb against transferrin receptor (95). This particular mAb 83-14, which is very much analogous to neuroactive chemicals, has a very strong affinity towards its targeting receptors and its transportation through brain is very easy.

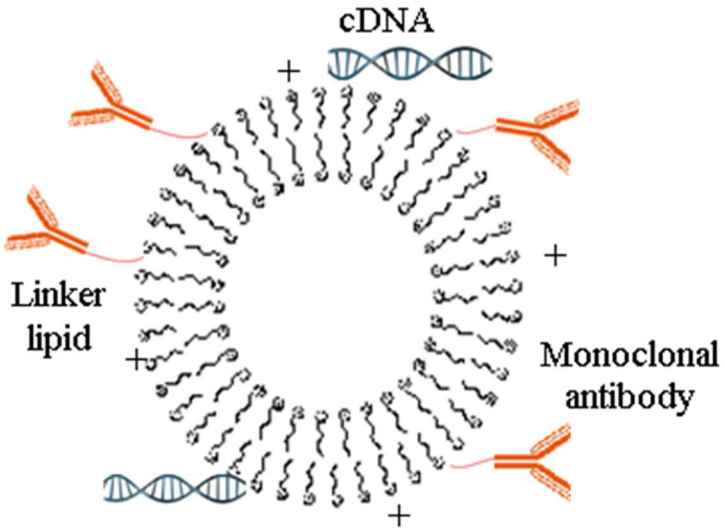

Figure 1. 5 Graphical representation of mAb conjugated liposomes complexed with cDNA

Liposomes Functionalization for ligand attachment. A variety of methods for coupling targeting ligands to the surface of preformed liposomes is descried below.

Classical Reactions to Modify Preformed Liposomes with examples

- Amine-Functionalized Liposomes:

  Amine containing natural lipids phosphatidylethanolamine like DSPE were used in primary studies for the functionalisation of liposomes to explore the intravascular targeted immunoliposomes. (16) The amine functional group containing egg or soya lecithin, cholesterol and phosphatidylethanolamine in a molar ratio of 7:2:1 and are reactive towards the ligands with amine functional group with proper linker. One usual tactic is to use imine or imidoester crosslinker, after reacting amine functionalised liposomes or ligand containing amine respectively with dimethyl suberimidate or glutaraldehyde. A number of targeting moiety has been attached to nanocarrier with this tactic. The biggest disadvantage of this particular method is the homobifuntionality (reaction of ligand with ligand or amino containing liposomes with themselves) of the used linkers which are to be used in large amount leading towards the side products and loss of targeting efficiencies (65).

- Carboxylic Acid-Functionalized Liposomes

  To overcome the clearance problem of one antithrombic agent, D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone short peptide (PPACK), the approach of amine-carboxylic acid conjugation was used on prepared liposomal surface. Pakeker et al. established that the above mentioned peptide PPACK can be

conjugated to already formed liposomal surface containing egg PC, DPPE, and DSPE-PEG_{2000}-COOH (95: 3: 2) by applying usual NH2-COOH coupling conditions for the conjugation of amine containing peptide and preformed COOH terminated DSPE-PEG-encompassing liposomes. By this approach, the peptide was attached to small unilamellar liposomes and they were separated from unconjugated portions using dialysis technique for around 4 h, which also indicated the stability of formed system. The change in zeta potential values indirectly indicated the peptide conjugation to liposomal formulation during the experiment (96). The liposomes for thrombin targeting indicated a potent antithrombin effect however after the intravenous injection of the same, targeted liposomes were found to be accumulated in liver almost after 2 h and showed detrimental effects. Even though the accurate half-lifefor the newly formed liposomes were not stated but *in vivo* results propose improved half-life over the original half-life of naked peptide.

- para-Nitrophenylcarbonyl-Functionalized Liposomes

  10-15 years back, Torchilin et al. familiarised us with the alternate technique to attach proteins or antibody to the liposomes in one step with very high efficiency. A new derivative of DOPE, para-nitrophenylcarbonyl (pNP)-PEG-DOPE was introduced and used in the preparation of pNP attached liposomes. The liposomes were formulated using thin film hydration method using Egg PC and cholesterol in 7 : 3 molar ratio with varied amount of modified DOPE which was hydrated using citrate buffer of pH 5.1 to avoid hydrolysis of pNP. This synthesised pNP can react with primary amine in buffer of pH ranging from 8 to 10, results in liposome-protein system of nano size. This modified or synthesised lipid required to be used in minimum quantity around 0.5 mole % of total lipids used in formulation to conjugate protein to be targeted to the liposomes and the unused pNP molecules get hydrolysed to evade any undesired reactions, these two are the main advantages linked with the use of this particular linker. Additionally, unlike other reactions, there is no need to active carboxylic acid to react with amine group, and in a way it decreases the time required for finishing the complete reaction.Alternatively, to avoid the hydrolysis of pNP befoe completing the reaction, it should be maintained at acidic pH. To evaluate the stability of liposome conjugated with antibody through pNP, the same liposome was loaded with calcein and incubated with mouse serum. Calcein was used in a self-

quenching concentration to load in liposomes and the fluorescence after its leakage form liposomes, at various time points(97). Liposomes comprising of pNP-PEG-DOPE exhibited same stability when incubated with mouse serum, with and without conjugated to monoclonal antibofy 2C5 while the liposomes without the use of pNP-PEG-DOPE showed less promising results under same environment. In some another research, TAT peptide was effectively reacted with pNP-PEG-DOPE on the preformed liposomes (98). Though the stability data and zeta potential data for the same study was missing which was very crucial for the experiment as the TAT peptide contains very high positive charge. But the results proved that the conjugation of mAb 2C5 or TAT peptide to the liposomes maintained the specific targeting efficacy of used ligands. The chances of changing the active confirmation form of targeting moiety was considered during the design of experiment and evaluation stage of targeted system.

- A protein-bound dithiopyridine (protein-DTP)

  Protein or antibody bound with DTP can be activated at lower pH to react with another molecule containing DTP modified liposomes to create a bridge containing disulphide bond which offers the prospect to use DTP to conjugate antibody to liposomes comprising of thiol reactive groups. The principleadvantage of this particular method is that the reaction can be supervised spectroscopically with the release of a 2-2-thiopyridone chromophore group. Commonly one such linker using the disulphide bridge to conjugate antibody to amine containing liposome is SPDP (3-(2-pyridyldithio) propionate). Liposomes conjugated with fragmented antibodies Fab' showed not more than 7 h of stability at physiological pH but the stability in serum was even less because of the presence of reductants present in serum can easily readuce the disulphide bond and free the antibody attached on the surface of liposomes (99).

  Liposomes targeted with Fab' antibody fragments appeared to be stable at physiological pH for longer than 7 h; nevertheless, the stability in serum was decreased, most likely due to the presence of biological reductants reducing the disulfide bond and releasing the antibody from the surface of the liposomes.

- Maleimide-Functionalized Liposomes.

  DSPE functionalised with maleimide or bromoacetyl group, was synthesised and used in the preparation of liposomes formulated from HSPC, DSPG and

cholesterol in molar ratio of 10 : 65: 25. Thiol containing moieties are reactive towards both the meleimide and bromoacetyl derivative of DSPE. Schelté et al. studied the conjugation efficacy of thiol containing protein or antibody with the liposomes containing DSPE functionalised meleimide or bromoacetyl derivative. The main difference between maleimide and bromoacetyl derivative pf DSPE is that they both react with thiols at different pH (maleimide reacts at pH 6.5 and bromoacetyl at 9.0), signifying the importance of using both the derivatives together for antibody containing thiols in different environment to react with liposomes.

- DSPE-PEG-maleimide

  This commercially available phospholipid linker has been extensively used for the functionalization of liposomes with thiol-containing ligands. Lately, H2009.1 peptide in its monomeric and tetrameric forms were used to target integrin present on the surface of non-small cell lung cancer cells, with their attachment to liposomes comprised of DSPE-PEG_{2000}-maleimide as a part of their formula. Three different liposomal formulations with varied molar ratios of DSPE-PEG_{2000}-maleimide to other lipids were formed and analysed. HSPC and cholesterol (65: 32) were dissolved in chloroform: methanol mixture and three different films were formed using DSPE-PEG_{2000} and DSPE-PEG_{2000}-maleimide in different molar ratios of .5: 0.64, 1.9: 1.3, or 1.2: 2.During the loading of doxorubicin, the hydration of lipid film was achived using ammonium sulfate buffer (pH 5.5) at the temperature of around 60-65 °C, and the resulting dispersion was passed through the extrusion to get liposomes below the size of 100 nm and the used ammonium buffer was exchanged with citrate buffer of same pH. Later on, the monomeric and tetrameric peptides having a cysteine moiety were attached to liposomal surface through this coupling reaction involving maleimide-thiol mechanism with dissolving excess amount of peptide in HEPES buffer. The efficacy of peptide conjugation to liposomal surface was found to be around 90 % depending on the peptide stationed on the outer surface of the liposomes and peptide was added to the liposomal dispersion after the maleimide present on the liposome surface was quenched using mercaptohanol. As soon as the maleimide reacted with mercaptoethanol, the prefromed liposomes cannot conjugate with added peptide. Even after the 24 h of incubation of peptide with liposomes not resulting in peptide attachment to liposomes is the proof of peptide is not conjugated to

liposomal vesical in spontaneous manner, which is an important parameter for identification of integrin present on the cell surface. The effects of monomeric and tetrameric peptides with +2 and +4 charge respectively, was investigated and owing to their different charge densities they have varied tendencies for cell uptake. The experiments carried out in *in vitro* set up, showed a cellular uptake of H2009.1 attached liposomes consistent with the charge present on their surface. Even though holding same charge and same amino acid chains, the scramble peptide scH2009.1, when conjugated to liposomes, cannot specifically bind to integrin receptors on cell surface, the way H2009.1 can able to identify integrin receptors. The results also showed d that the tetrameric peptide H2009.1 attached to liposomes, showed increased binding capacity in comparison to scH2009.1 tetrameric peptide, presenting re captor specific binding as well as nonspecific binding induced by surface charge. The outcome of peptide charge on the zeta potential of liposomal system was not analysed yet. Furthermore, there was no study indicating the leakage of doxorubicin from the liposomal vesicle was found. Moreover, confocal microscopy on non-small cell lung cancer containing integrin receptors showed that the liposomes were totally intact when they were found in extracellular matrix and the drug release took place only when the liposomes entered the cells, signifying only minor leakage of liposomal content took place under the physiological conditions.

- Aldehyde-Functionalized Liposomes

  Two decades ago, Bourel-Bonnet et al. showed a new technique for ligand conjugation established by the hydrazine bond formation between derivative of α-oxoaldehyde palmitoyl and peptide containing α-hydrazine acetyl group. This tactic shows many advantages including the generation of no other side products other than water which is easily removable moreover, reaction can be accomplished under mild conditions. Furthermore, the macromolecules are devoid of functional group that can ultimately form hydrazine bond rendering the conjugation process regioselective (48).

  Additional studies regarding thermodynamics and kinetic parameters for the conjugation using hydrazine bonding was reported later on which proves ligation as quantitative and more of a selective process in nature. Besides, when this conjugation process was carried out in colloidal dispersion under physiological conditions the stability of ligation was increased due to autoassociation of all the

reagents used in the process however this hydration bond is less stable in solutions other than colloidal media. Numerous peptidoliposomes (peptide conjugated liposomes) were prepared using this approach and results confirmed the usefulness of this technique in targeting.

1.12.2 Intranasal delivery

Intranasal delivery offers a method to deliver polynucleotide agents, specially ssDNA or siRNA (oligonucleotides) to the brain by using neural pathway starting from intranasal cavity or from an extranasal tissue which is innervated by the trigeminal nerves. The death risk attached with CNS diseases is one of the major health issue in the world. With the use of polynucleotide agents such as plasmid DNA or siRNA have been known to solve these problems but the main problem linked to such agents is their delivery to the site of action at doses i.e. therapeutically effective for the treatment. Nevertheless in last decade there have been many researches that showed the effective use of polynucleotide agents to manipulate protein level in brain for the successful treatment of brain disorder. Many reports have been submitted involving gene modifications for receptors in brain for the neurotransmitter, cytokines, transporters and other important proteins.

Orthodox methods for delivery of drug to brain comprise: neurosurgical approach which includes intracerebral injection or infusion for intracerebroventricular route; modification of therapeutic agents which include production and engineering of proteins created through joining of more than two or three genes also known as chimeric protein which usually involves the protein useful for transportation through BBB in combination with agents useful for therapeutic effects; considerate strategies that improve the lipid solubility of therapeutic agents which include hydrophilic or water soluble agents to be conjugated with lipids or acyl chain and use of hyperosmotic disruption method to disturb the BBB structure following the infusion of sugar such as mannitol solution in the carotid artery or by using therapeutically active gents including angiotensin peptides).Yet most of these tactics are not devoid of limitations like integral issues including death risk is involved with invasive surgery to deposit therapeutic gene in brain, limitation of particle size imposes many intrinsic problem due to the endogenous system to transport molecules to the other side of brain, possibly many side effects are linked with the parenteral administration of chimeric or fused proteins which may have effects even outside the brain area, having peripheral undesirable effects or efficacy for more than one receptors in brain resulting in CNS side effects for such agents. They may also result in damage of brain cells or nerves in the area of disrupted BBB which reduce these methods to a less effective

delivery methods (29). Also the inner regions of brain also separated from each other by white matter which is hydrophobic in nature, therefore direct injection to cerebral area also cannot promote distribution of therapeutic agents in whole brain area. This results in need of a better way of delivering therapeutic agents to the brain tissues and cells present there. This need results in delivery of polynucleotide as therapeutic agents through the neuronal pathway of nasal cavity as an alternative to systemic delivery for the same. By avoiding the problem of penetrating BBB, the delivery though nasal cavity averts the problems forced by BBB helps transporting the polynucleotide agents that are poorly transported before through systemic delivery or completely unable to penetrate BBB. This nasal cavity not only increase the transportation efficiency but also decreases the therapeutic dose required for the treatment of disease. This decrease in dose simultaneously lead to minimal undesired side effects associated with systemic delivery of therapeutic agents. More precisely, the intranasal route delivers the polynucleotide agents through the nasal cavity using neuronal pathway linked with olfactory or trigeminal nerves. The one-third part of nasal cavity where the olfactory region is situated. As an alternative to this olfactory region, the therapeutic agents can also be delivered to tissues comprising of trigeminal nerves.

Transportation through neural pathway or by the way of neuronal pathway comprises of intracellular route for axonal transportation; extracellular route for transportation using clefts present between cells of olfactory region; transportation through or with the help of neuronal based endocytosis involving fluid phase. Perivascular space which is a space between blood vessels and neurons connected to them; lymphatic channels linked with neurons also helps in transportation. Hemangiolymphatic system, epithelial cell layer and mucosal layer, also play significant role in this transportation through neuronal pathway (100).

To deliver the polynucleotides to the brain, they are administered to or through the olfactory pathway initiating from olfactory region between nasal septum and side walls of each side of nasal cavity. If possible the formulation is delivered through the upper third portion of nasal cavity or in other words though the olfactory epithelium. Formulation admisitered in this olfactory region can take as aforementioned any extracellular or intracellular route to end uo in brain. For instance, the molecule may transported to brain all along the olfactory nerve or it pass though it which is olfactory neuronal pathway or through lymphatic system associated with olfactory neurons which is also known as hemangiolymphatic system. An alternative pathway to this olfactory region to transport

molecules to the brain is by using trigeminal pathway making its way from the trigeminal nerves (101) . Appropriate tissues comprise of both the intranasal and extra nasal tissues that are the originating points for ophthalmic nerve, maxillary nerve, and mandibular nerve of trigeminal nerve.

Use of neuronal pathways, for the transportation of various kind of molecules to the brain avoids the hurdles due to BBB and permits most classes of therapeutic agents such ssDNA, siRNA, chimeric antisense molecules. Even though the above mentioned therapeutic molecules may get absorbed into blood circulation, the specific sequence characteristic of such agents will minimise the chances of possible systemic side effects. Moreover, the agents to be administered using this route are not get diluted with blood present in circulation, the higher concentration of therapeutic agents can be achieved in brain cells and tissues than with parenteral route.

References

1. Chambless DL, Ollendick TH. Empirically supported psychological interventions: Controversies and evidence. Annual review of psychology. 2001;52(1):685-716.
2. Organization WH. Global and regional estimates of violence against women: prevalence and health effects of intimate partner violence and non-partner sexual violence: World Health Organization; 2013.
3. Hyde JS, Mezulis AH, Abramson LY. The ABCs of depression: integrating affective, biological, and cognitive models to explain the emergence of the gender difference in depression. Psychological review. 2008;115(2):291.
4. Dohrenwend BP. The role of adversity and stress in psychopathology: Some evidence and its implications for theory and research. Journal of health and social behavior. 2000:1-19.
5. Beck AT, Alford BA. Depression: Causes and treatment: University of Pennsylvania Press; 2009.
6. Teasdale JD, Segal ZV, Williams JMG, Ridgeway VA, Soulsby JM, Lau MA. Prevention of relapse/recurrence in major depression by mindfulness-based cognitive therapy. Journal of consulting and clinical psychology. 2000;68(4):615.
7. Lemoine P, Guilleminault C, Alvarez E. Improvement in subjective sleep in major depressive disorder with a novel antidepressant, agomelatine: randomized, double-blind comparison with venlafaxine. Journal of Clinical Psychiatry. 2007;68(11):1723-32.
8. Csoka A, Bahrick A, Mehtonen OP. Persistent sexual dysfunction after discontinuation of selective serotonin reuptake inhibitors. The journal of sexual medicine. 2008;5(1):227-33.
9. Whyte IM, Dawson A, Buckley N. Relative toxicity of venlafaxine and selective serotonin reuptake inhibitors in overdose compared to tricyclic antidepressants. Qjm. 2003;96(5):369-74.
10. Gillman P. Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. British journal of pharmacology. 2007;151(6):737-48.
11. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacology & therapeutics. 2007;116(3):496-526.

12. Elkin I, Shea MT, Watkins JT, Imber SD, Sotsky SM, Collins JF, et al. National Institute of Mental Health treatment of depression collaborative research program: General effectiveness of treatments. Archives of general psychiatry. 1989;46(11):971-82.
13. Association AP. Practice guideline for the treatment of patients with bipolar disorder (revision): American Psychiatric Pub; 2002.
14. Cherlyn SYT, San Woon P, Liu JJ, Ong WY, Tsai GC, Sim K. Genetic association studies of glutamate, GABA and related genes in schizophrenia and bipolar disorder: a decade of advance. Neuroscience & Biobehavioral Reviews. 2010;34(6):958-77.
15. Cryan JF, Slattery DA. Animal models of mood disorders: recent developments. Current opinion in psychiatry. 2007;20(1):1-7.
16. DeRubeis RJ, Hollon SD, Amsterdam JD, Shelton RC, Young PR, Salomon RM, et al. Cognitive therapy vs medications in the treatment of moderate to severe depression. Archives of general psychiatry. 2005;62(4):409-16.
17. Administrators C. MoPath: GeneSight Assay for Refractory Depression (L35437). MediQuant. com [Internet].
18. Smith RE, Tran K, Kristy M. Jacobs Journal of Medicinal Chemistry. 2015.
19. Woolf CJ. Overcoming obstacles to developing new analgesics. Nature medicine. 2010;16(11):1241.
20. Hepgul N, Cattaneo A, Zunszain PA, Pariante CM. Depression pathogenesis and treatment: what can we learn from blood mRNA expression? BMC Medicine. 2013;11(1):28.
21. Huang Y, Williams WA. Enhanced selective serotonin re-uptake inhibitors as antidepressants: 2004–2006. Expert opinion on therapeutic patents. 2007;17(8):889-907.
22. Leistedt SJ, Linkowski P. Brain, networks, depression, and more. European neuropsychopharmacology. 2013;23(1):55-62.
23. Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G, et al. A mega-analysis of genome-wide association studies for major depressive disorder. Molecular psychiatry. 2013;18(4):497.
24. Liu H-K, Schuetz G. Means and methods for treating or preventing brain tumors based on the nuclear receptor tailless (Tlx). Google Patents; 2013.
25. Lader M. Generalized anxiety disorder. Encyclopedia of Psychopharmacology: Springer; 2015. p. 699-702.

26. Duman RS, Aghajanian GK. Synaptic dysfunction in depression: potential therapeutic targets. science. 2012;338(6103):68-72.
27. Alagiakrishnan K, Gill SS, Fagarasanu A. Genetics and epigenetics of Alzheimer's disease. Postgraduate medical journal. 2012;88(1043):522-9.
28. Karp DA. Speaking of sadness: Depression, disconnection, and the meanings of illness: Oxford University Press; 2016.
29. Segal ZV, Williams JMG, Teasdale JD. Mindfulness-based cognitive therapy for depression: Guilford Press; 2012.
30. Gaiteri C. Finding the pathology of major depression through effects on gene interaction networks: University of Pittsburgh; 2011.
31. Alexander B, Warner-Schmidt J, Eriksson T, Tamminga C, Arango-Lievano M, Ghose S, et al. Reversal of depressed behaviors in mice by p11 gene therapy in the nucleus accumbens. Sci Transl Med. 2010;2(54):54ra76.
32. Patzelt H, Shepherd DA. Negative emotions of an entrepreneurial career: Self-employment and regulatory coping behaviors. Journal of business venturing. 2011;26(2):226-38.
33. L Neto F, Borges G, Torres-Sanchez S, A Mico J, Berrocoso E. Neurotrophins role in depression neurobiology: a review of basic and clinical evidence. Current neuropharmacology. 2011;9(4):530-52.
34. Warner-Schmidt JL, Flajolet M, Maller A, Chen EY, Qi H, Svenningsson P, et al. Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. Journal of Neuroscience. 2009;29(6):1937-46.
35. Krishnan V, Nestler EJ. Animal models of depression: molecular perspectives. Molecular and Functional Models in Neuropsychiatry: Springer; 2011. p. 121-47.
36. Marongiu R, Arango-Lievano M, Francardo V, Morgenstern P, Zhang X, Cenci MA, et al. Gene therapy blockade of dorsal striatal p11 improves motor function and dyskinesia in parkinsonian mice. Proceedings of the National Academy of Sciences. 2016;113(5):1423-8.
37. Green H, Zhang X, Tiklova K, Volakakis N, Brodin L, Berg L, et al. Alterations of p11 in brain tissue and peripheral blood leukocytes in Parkinson’s disease. Proceedings of the National Academy of Sciences. 2017:201621218.
38. Svenningsson P, Kim Y, Warner-Schmidt J, Oh Y-S, Greengard P. p11 and its role in depression and therapeutic responses to antidepressants. Nature Reviews Neuroscience. 2013;14(10):673.

39. Guo J, Zhang W, Zhang L, Ding H, Zhang J, Song C, et al. Probable involvement of p11 with interferon alpha induced depression. Scientific reports. 2016;6:17029.
40. Xu C. Impact of a Decision Support System on Obstetricians in the Pregnancy Nutrition Guidance. 2012.
41. Ruf B, Bhagwagar Z. The 5-HT1B Receptor: A Novel Target for the Pathophysiology of Depression (Supplementary Tables). Current drug targets. 2009;10(11):1118-38.
42. Zhang C, Lueptow LM, Zhang H-T, O'Donnell JM, Xu Y. The Role of Phosphodiesterase-2 in Psychiatric and Neurodegenerative Disorders. Phosphodiesterases: CNS Functions and Diseases: Springer; 2017. p. 307-47.
43. Bollen E, Prickaerts J. Phosphodiesterases in neurodegenerative disorders. IUBMB life. 2012;64(12):965-70.
44. Heckman P, Blokland A, Bollen E, Prickaerts J. Phosphodiesterase inhibition and modulation of corticostriatal and hippocampal circuits: Clinical overview and translational considerations. Neuroscience & Biobehavioral Reviews. 2018.
45. Horwitz AV. How an age of anxiety became an age of depression. The Milbank Quarterly. 2010;88(1):112-38.
46. Tamatam A, Khanum F, Bawa AS. Genetic biomarkers of depression. Indian journal of human genetics. 2012;18(1):20.
47. Fabbri C, Porcelli S, Serretti A. From pharmacogenetics to pharmacogenomics: the way toward the personalization of antidepressant treatment. The Canadian Journal of Psychiatry. 2014;59(2):62-75.
48. Hemeryck A, Belpaire FM. Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. Current drug metabolism. 2002;3(1):13-37.
49. Lapierre YD. Suicidality with selective serotonin reuptake inhibitors: Valid claim? Journal of Psychiatry and Neuroscience. 2003;28(5):340.
50. Laje G, Allen AS, Akula N, Manji H, Rush AJ, McMahon FJ. Genome-wide association study of suicidal ideation emerging during citalopram treatment of depressed outpatients. Pharmacogenetics and genomics. 2009;19(9):666.
51. Wong M-L, Whelan F, Deloukas P, Whittaker P, Delgado M, Cantor RM, et al. Phosphodiesterase genes are associated with susceptibility to major depression and antidepressant treatment response. Proceedings of the National Academy of Sciences. 2006;103(41):15124-9.

52. Vaeth PA, Caetano R, Mills BA. Factors associated with depression among Mexican Americans living in US–Mexico border and non-border areas. Journal of immigrant and minority health. 2016;18(4):718-27.
53. Luo H-R, Wu G-S, Dong C, Arcos-Burgos M, Ribeiro L, Licinio J, et al. Association of PDE11A global haplotype with major depression and antidepressant drug response. Neuropsychiatric disease and treatment. 2009;5:163.
54. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. Advanced biomedical research. 2012;1.
55. Hardee CL, Arévalo-Soliz LM, Hornstein BD, Zechiedrich L. Advances in non-viral DNA vectors for gene therapy. Genes. 2017;8(2):65.
56. Howarth JL, Lee YB, Uney JB. Using viral vectors as gene transfer tools (Cell Biology and Toxicology Special Issue: ETCS-UK 1 day meeting on genetic manipulation of cells). Cell biology and toxicology. 2010;26(1):1-20.
57. Pozzi D, Marchini C, Cardarelli F, Amenitsch H, Garulli C, Bifone A, et al. Transfection efficiency boost of cholesterol-containing lipoplexes. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2012;1818(9):2335-43.
58. Lam JK, Chow MY, Zhang Y, Leung SW. siRNA versus miRNA as therapeutics for gene silencing. Molecular Therapy-Nucleic Acids. 2015;4.
59. Lynch JW. Molecular structure and function of the glycine receptor chloride channel. Physiological reviews. 2004;84(4):1051-95.
60. Bouard D, Alazard-Dany N, Cosset FL. Viral vectors: from virology to transgene expression. British journal of pharmacology. 2009;157(2):153-65.
61. Spagnou S, Miller AD, Keller M. Lipidic carriers of siRNA: differences in the formulation, cellular uptake, and delivery with plasmid DNA. Biochemistry. 2004;43(42):13348-56.
62. Abe A, Shayman JA. The role of negatively charged lipids in lysosomal phospholipase A2 function. Journal of lipid research. 2009;50(10):2027-35.
63. Uddin SN. Cationic lipids used in non-viral gene delivery systems. Biotechnology and Molecular Biology Reviews. 2007;2(3):058-67.
64. Grazu V, Moros M, Sánchez-Espinel C. Nanocarriers as nanomedicines: design concepts and recent advances. Frontiers of Nanoscience. 4: Elsevier; 2012. p. 337-440.
65. Marqués-Gallego P, de Kroon AI. Ligation strategies for targeting liposomal nanocarriers. BioMed research international. 2014;2014.

66. Balazs DA, Godbey W. Liposomes for use in gene delivery. Journal of drug delivery. 2011;2011.
67. Rasoulianboroujeni M, Kupgan G, Moghadam F, Tahriri M, Boughdachi A, Khoshkenar P, et al. Development of a DNA-liposome complex for gene delivery applications. Materials Science and Engineering: C. 2017;75:191-7.
68. Jin L, Zeng X, Liu M, Deng Y, He N. Current progress in gene delivery technology based on chemical methods and nano-carriers. Theranostics. 2014;4(3):240.
69. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. The transport of molecules between the nucleus and the cytosol. 2002.
70. Leonhardt C, Schwake G, Stögbauer TR, Rappl S, Kuhr J-T, Ligon TS, et al. Single-cell mRNA transfection studies: delivery, kinetics and statistics by numbers. Nanomedicine: Nanotechnology, Biology and Medicine. 2014;10(4):679-88.
71. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proceedings of the National Academy of Sciences. 1987;84(21):7413-7.
72. Cervia LD, Chang C-C, Wang L, Yuan F. Distinct effects of endosomal escape and inhibition of endosomal trafficking on gene delivery via electrotransfection. PloS one. 2017;12(2):e0171699.
73. Felgner JH, Kumar R, Sridhar C, Wheeler CJ, Tsai YJ, Border R, et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. Journal of Biological Chemistry. 1994;269(4):2550-61.
74. Kreiss P, Mailhe P, Scherman D, Pitard B, Cameron B, Rangara R, et al. Plasmid DNA size does not affect the physicochemical properties of lipoplexes but modulates gene transfer efficiency. Nucleic acids research. 1999;27(19):3792-8.
75. Majzoub RN, Chan C-L, Ewert KK, Silva BF, Liang KS, Jacovetty EL, et al. Uptake and transfection efficiency of PEGylated cationic liposome–DNA complexes with and without RGD-tagging. Biomaterials. 2014;35(18):4996-5005.
76. Pozharski E, MacDonald RC. Lipoplex thermodynamics: determination of DNA-cationic lipoid interaction energies. Biophysical journal. 2003;85(6):3969-78.
77. Scarzello M, Chupin V, Wagenaar A, Stuart MC, Engberts JB, Hulst R. Polymorphism of pyridinium amphiphiles for gene delivery: influence of ionic strength, helper lipid content, and plasmid DNA complexation. Biophysical journal. 2005;88(3):2104-13.

78. Felgner PL, Ringold G. Cationic liposome-mediated transfection. Nature. 1989;337(6205):387.
79. Stokes A, Wilson J, Warren W. Characterization of restricted diffusion in uni-and multi-lamellar vesicles using short distance iMQCs. Journal of Magnetic Resonance. 2012;223:31-40.
80. Zhang X-X, McIntosh TJ, Grinstaff MW. Functional lipids and lipoplexes for improved gene delivery. Biochimie. 2012;94(1):42-58.
81. Deuling H, Helfrich W. The curvature elasticity of fluid membranes: a catalogue of vesicle shapes. Journal de Physique. 1976;37(11):1335-45.
82. Campen RK, Ngo TT, Sovago M, Ruysschaert J-M, Bonn M. Molecular restructuring of water and lipids upon the interaction of DNA with lipid monolayers. Journal of the American Chemical Society. 2010;132(23):8037-47.
83. Wasungu L, Hoekstra D. Cationic lipids, lipoplexes and intracellular delivery of genes. Journal of Controlled Release. 2006;116(2):255-64.
84. Dass CR, Su T. Particle-mediated intravascular delivery of oligonucleotides to tumors: associated biology and lessons from genotherapy. Drug Delivery. 2001;8(4):191-213.
85. May S, Ben-Shaul A. Modeling of cationic lipid-DNA complexes. Current medicinal chemistry. 2004;11(2):151-67.
86. Huebner S, Battersby BJ, Grimm R, Cevc G. Lipid-DNA complex formation: reorganization and rupture of lipid vesicles in the presence of DNA as observed by cryoelectron microscopy. Biophysical journal. 1999;76(6):3158-66.
87. Zhi D, Zhang S, Cui S, Zhao Y, Wang Y, Zhao D. The headgroup evolution of cationic lipids for gene delivery. Bioconjugate chemistry. 2013;24(4):487-519.
88. Du Z, Munye MM, Tagalakis AD, Manunta MD, Hart SL. The role of the helper lipid on the DNA transfection efficiency of lipopolyplex formulations. Scientific reports. 2014;4:7107.
89. Shokri M, Tavallaie M, Hosseini SM. Effect of Lipoplex Charge Ratio on Transfection Efficiency of Osteosarcoma Cells. 2016.
90. Saucier-Sawyer JK, Deng Y, Seo Y-E, Cheng CJ, Zhang J, Quijano E, et al. Systemic delivery of blood–brain barrier-targeted polymeric nanoparticles enhances delivery to brain tissue. Journal of drug targeting. 2015;23(7-8):736-49.

91. Luissint A-C, Artus C, Glacial F, Ganeshamoorthy K, Couraud P-O. Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. Fluids and Barriers of the CNS. 2012;9(1):23.
92. Moos T, Morgan EH. Transferrin and transferrin receptor function in brain barrier systems. Cellular and molecular neurobiology. 2000;20(1):77-95.
93. Apiratmateekul N, Phunpae P, Kasinrerk W. A modified hybridoma technique for production of monoclonal antibodies having desired isotypes. Cytotechnology. 2009;60(1-3):45.
94. Ma H, Zhang T, Shen H, Cao H, Du J. The adverse events profile of anti-IGF-1R monoclonal antibodies in cancer therapy. British journal of clinical pharmacology. 2014;77(6):917-28.
95. Wu D, Pardridge WM. Pharmacokinetics and blood-brain barrier transport of an anti-transferrin receptor monoclonal antibody (OX26) in rats after chronic treatment with the antibody. Drug metabolism and disposition. 1998;26(9):937-9.
96. Guo X, Wu Z, Guo Z. New method for site-specific modification of liposomes with proteins using sortase A-mediated transpeptidation. Bioconjugate chemistry. 2012;23(3):650-5.
97. Torchilin V, Levchenko T, Lukyanov A, Khaw B, Klibanov A, Rammohan R, et al. p-Nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2001;1511(2):397-411.
98. Apte A, Koren E, Koshkaryev A, Torchilin VP. Doxorubicin in TAT peptide-modified multifunctional immunoliposomes demonstrates increased activity against both drug-sensitive and drug-resistant ovarian cancer models. Cancer biology & therapy. 2014;15(1):69-80.
99. Sirich TL, Meyer TW, Gondouin B, Brunet P, Niwa T, editors. Protein-bound molecules: a large family with a bad character. Seminars in nephrology; 2014: Elsevier.
100. Djupesland PG, Mahmoud RA, Messina JC. Accessing the brain: the nose may know the way. Journal of Cerebral Blood Flow & Metabolism. 2013;33(5):793-4.
101. Chapman CD, Frey WH, Craft S, Danielyan L, Hallschmid M, Schiöth HB, et al. Intranasal treatment of central nervous system dysfunction in humans. Pharmaceutical research. 2013;30(10):2475-84.