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An Overview On Tyrosine Kinase Inhibitor Gefitinib Drug Delivery Systems For Enhanced Treatment Of Cancer

Pedireddi Sobitha Rani[1]*, Singireddy Anand Reddy[2], Kususma Anusha[4], Pola Kranthi Kumar[3] and Pulla Udaya Chanrika[1]

[1]Bhaskar Pharmacy College, Bhaskar Nagar, Yenkapally, Moinabad, Hyderabad, Telangana, India.
[2]Vijaya College of Pharmacy, Munaganoor, Hayath Nagar, Hyderabad, Telangana, India.
[3]Balaji Institute of Pharmaceutical Sciences, Laknepally, Narsampet, Warangal, Telangana, India.
[4]Joginapally B.R. Pharmacy College, Bhaskar Nagar, Yenkapally, Moinabad, Hyderabad, Telangana, India.

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IRESSA (gefitinib), an oral drug used once daily at a dose of 250 mg, suppresses the activity of the epidermal growth factor receptor tyrosine kinase (EGFR-TK). Having been granted a license for the first EGFR-TK inhibitor to treat non-small cell lung cancer, it is presently acknowledged in over 70 countries worldwide. Extensive research has been devoted to examining its beneficial impacts on human health, encompassing anti-oxidant, anti-inflammatory, cardioprotective, and anti-tumor properties. The development of therapeutic applications is hampered by the low bioavailability (BA) of gefitinib, which appears to be a severe limitation on the drug's in vivo biological effects. Within this particular framework, a growing sum of current research has focused on developing innovative gefitinib formulations to get beyond their low solubility, restricted stability, excessive metabolization, and inadequate BA. This study addresses the physicochemical and pharmacokinetic (PK) constraints on gefitinib BA, discusses the preparations that have been tried for the administration, controlled release, and targeting of gefitinib, and pinpoints potential forthcoming routes for gefitinib delivery.

Keywords: Administration Routes; Cystic fibrosis; Dry powder inhalers; Lung infections; Pharmacokinetics; Wetness Impregnation Method; Tyrosine Kinase.

Gefitinib (IRESSA, ZD1839), a low-molecular-weight anticancer medication, inhibits the tyrosine kinase enzyme to stop EGFR signaling in target cells [1]. Ovarian, colon, breast, pancreatic, and lung tumors are treated with it [2]. It is the first-line treatment for EGFR-mutated Non-Small-Cell Lung Cancer (NSCLC) patients. This precision targeting disrupts cancer cell proliferation and survival pathways, making it a valuable therapeutic option. However, its clinical application is hindered by several limitations, including poor aqueous solubility, low oral bioavailability, rapid clearance, and the emergence of resistance in patients. These challenges have prompted significant research into advanced drug delivery systems to enhance Gefitinib's therapeutic potential and patient outcomes. The Food Drug Administration (FDA) authorized it in 2015 [3]. With the chemical name 4-Quinazolinamine, N-(3-chloro-4-fluorophenyl) -7-methoxy-6-[3-4-morpholin] propoxy, it is an anilinoquinazoline.

*Corresponding author E-mail: sobhitarani@gmail.com

The aim of this review article is to provide a comprehensive analysis of Gefitinib, and its role in cancer therapy, highlighting its therapeutic potential and limitations such as poor solubility, low bioavailability, and resistance development. The article seeks to explore advancements in drug delivery systems, including nanotechnology, liposomal formulations, and polymer-based systems, that address these challenges and enhance the drug's pharmacokinetic and pharmacodynamic profiles. Additionally, it aims to evaluate the clinical implications of these innovative approaches in improving the efficacy and safety of Gefitinib for cancer treatment.

Physicochemical properties of gefitinib

The substance is a white powder with the chemical formula $C_{22}H_{24}ClFN_4O_3$ with a relative molecular mass of 446.9 g/mol. The compound exhibits high solubility in Dimethyl sulfoxide (DMSO) and glacial acetic acid, moderate solubility in pyridine, low solubility in tetrahydrofuran, and limited solubility in ethyl acetate, methanol, propane-2-ol, ethanol, and acetonitrile [4]. The Biopharmaceutical Classification System (BCS) classifies it as a class II molecule due to its low intestinal solubility, dissolution rate, and high permeability [5]. It has a low solubility at pH 1 and a precipitous drop in solubility between pH 4 and 6, in particular. That changes the BA when it starts working, and how well it works as a medicine. Because of its dibasic nature and high hydrophobicity (log P-value=4.15), gefitinib ionizes in solution at a rate proportional to the decrease in pH [6]. Many nations have authorized gefitinib, and the recommended daily dosage is 250 mg [7]. This includes the United States, Japan, and Australia. The following adverse drug reactions are commonly reported with this dosage: anorexia, skin and subcutaneous disorders, hepatobiliary disorders, gastrointestinal (GI) disorders, and more. These include disorders of metabolism and nutrition, anorexia, skin reactions, pustular rash, dry skin, and itching [8]. These issues highlight the necessity to enhance the oral BA of gefitinib and decrease the daily oral dose. An increase in the drug's solubility and dissolving power could raise the oral BA for this dose from 57% to 58% [9]. Various approaches like micronization of drug particles, solvent dispersion on an inert carrier, hot-melt extrusion, nanoparticle formation, and cyclodextrin complexation can be followed to enhance the solubility and dissolution of gefitinib. This permits a decrease in dosage and adverse effects associated with oral dosage [4,10].

A dose-dependent grade 3 or 4 rash may develop on days 10–14 of treatment. Additional skin side effects may manifest as dry skin, pruritis erythema, pustular or acne-like lesions, or both. The onset of dose-related diarrhea usually happens between days 8 and 14 of treatment. Interstitial pneumonia is the most severe side effect, occurring in 1% to 2% of patients treated with gefitinib [1,11]. Gefitinib, like many tyrosine kinase inhibitors, has a great volume of distribution and a half-life ($t_{1/2}$) of about 24-48 hours [12]. The parent drug and metabolites are primarily eliminated by the hepatic route; the renal route accounts for less than 4% of the dose [13]. Peak plasma levels of gefitinib in cancer patients after single-dose oral treatment occurred within 3-7 hours. Gefitinib has 60% BA. Meals heavy in fat-enhanced gefitinib exposure. Gefitinib has linear kinetics at therapeutic doses. The administration of a 50 mg intravenous bolus dose facilitates the systemic distribution of gefitinib, resulting in an average steady-state volume of distribution of 1400 liters. The mean total plasma clearance and elimination $t_{1/2}$ of gefitinib after a 50 mg i.v. bolus dosage was 595 ml/min and 48 hr, respectively. After 10 days of taking oral doses every day, the steady-state plasma levels were twice as high as those observed after taking individual doses. Total gefitinib binding to human plasma protein was 91%. Cytochrome P (CYP) 3A4 metabolizes gefitinib substantially in the liver. Although it is metabolized in the kidneys, only 4% of the supplied dose is eliminated through the kidneys. About 86% of the excretion is in the form of feces. There is no sign of dosage dependence, and values obtained from single doses or repeated administration are comparable [2]. The primary method by which Gefitinib exerts its anticancer properties is by competitively binding Mg-Adenosine triphosphate (ATP) on the catalytic domain of EGFR-TK [4]. This will prevent mitogen-activated protein kinase from activating and cause cancer cells to undergo apoptosis. Despite having a possible clinical use, Gefitinib has a large body distribution and causes severe drug-related toxicity. Additionally, a lack of medication availability in cancer cells makes chemotherapy treatment

more difficult [5]. To improve the chemotherapeutic efficacy of Gefitinib, lessen its toxicity, and raise its BA, new tactics must be developed [14].

PK key attributes of gefitinib

Many *in vitro* and *in vivo* research or academic investigations have outlined the PK distinctive attributes or qualities of this medication to pinpoint its absorption, metabolism, and ensuing BA. The *in vivo* outcome of gefitinib subsequent oral management has been recreated using data gathered from several *in vitro* cell culture models, models of small intestines isolated ex-vivo, animals studied *in vivo* and human subjects [16].

Intestinal absorption and metabolism

Ranson and group in 2002 conducted Preclinical toxicology studies in humans and demonstrated its good oral BA i.e., 57% (90% confidence interval, 49% to 68%) after 250mg dose and acceptability of a single and multiple doses of gefitinib. They also revealed the PK of the drug. Their tests found that gefitinib was slowly absorbed, unaffected by food intake, reached its peak plasma concentration 3-7 hours after oral dose, and reached steady-state concentration within the first week of once-daily therapy. The protein binding of gefitinib was 90% and was independent of the plasma concentration. Drug elimination occurs via the hepatobiliary pathway (86% in feces, < 4% in urine) with a $t_{1/2}$ of 28 hours, suggesting once-daily oral dosing is suitable. They found that gefitinib was well tolerated at 100 mg/day with few minor, temporary side effects [17]. Wilson and their group, studied how the GI transit factors affect the PKs of gefitinib in 12 Twelve male subjects (age range 28–60 years). They divided these 12 patients into 2 groups, Group A: normal profile and Group B: rapid clearance profile. In their studies, they found that the majority of subjects showed the elimination pattern of monophasic rather than biphasic. Group B patients had a faster mean gastric emptying $T_{90,}$ smaller intestinal transit time, and earlier colonic arrival time. The statistics suggested that GI transit characteristics are crucial in the rapid clearance profile group and contribute to the biphasic to monophasic changeover [18]..

In mice bearing intracerebral tumors, Jyoti Sharma. analyzed the intra-tumoral PK / pharmacodynamics (PD) characteristics of gefitinib. There was a 1.5-fold difference in Area Under the Curve (AUC) (0-24 h) between the highest and lowest gefitinib concentrations, and intra-tumoral drug concentration variability was 1.2- to 2.4-fold over 24 hours. Tumors were sampled at 150 mg/kg. Within a day, the PDs changed from 1.2 to 1.4-fold [19]. In 2013, Yan Chen and team, conducted a study to assess the ability of gefitinib to effectively traverse the blood-brain barrier (BBB). The researchers employed a mouse model of non-small-cell lung cancer with brain metastases to analyze the PKs and PDs of gefitinib. Using an *in vitro* MDCK-MDR1 assay, they calculated gefitinib's permeability and efflux ratio. The efflux ratio was found to be 4.12 and 4.05 at the concentrations of 1M and 10M respectively. $AUC_{total\ brain}/AUC_{total\ blood}$ was 0.4 in the 50 mg/kg group and 0.7 in the 200 mg/kg group in the BM model, whereas $AUC_{CSF}/AUC_{free\ blood}$ was 0.21 and 0.18, respectively [20]. In 2015, Yamaguchi, gave a 72-year-old continuous ambulatory peritoneal dialysis (CAPD) patient with non-small cell lung cancer 250mg of gefitinib daily. After giving gefitinib (626.6 ng/ml), the plasma concentration reached a steady state by day 16, was 538.4 ng/ml on day 46, and the peritoneal dialysis fluid concentration was 34.6 ng/ml, demonstrating that CAPD had little effect on gefitinib PKs [21].

Hepatic uptake and metabolism

Roberta Alfieri, examined the intracellular metabolism of gefitinib in two cell lines (gefitinib-sensitive and –resistant) to determine how CYP1A1 inhibition affects drug efficacy and found a significant difference. Detecting gefitinib metabolites within and outside sensitive cells showed that they metabolized the medication after 12-24 hours. CYP1A1-triggered gefitinib metabolism in lung cancer cells may indicate NSCLC cell responsiveness without activating mutations. However, inhibiting CYP1A1 in metabolizing cells may increase local drug exposure and gefitinib potency [22].

Distribution and excretion

It has been documented that gefitinib's PKs and long-term anticancer efficacy in people with lung adenocarcinomas who have the EGFR mutation are related. In this regard, partial response lung cancer patients receiving gefitinib displayed a lesser peak plasma Concentration (C_{max}) than subjects with stable disease. The $(AUC)_{0-24}$ of gefitinib, on the other hand, was found to have a significant negative connection with prolonged

survival [23]. Gefitinib is a drug that is widely absorbed after oral administration. A plasma elimination $t_{1/2}$ of 7 to 14 hours was recorded in rats and dogs after receiving an IV dose of gefitinib (5 mg/kg), with 1400 L apparent distribution volume [24]. Gefitinib is 90% bound to á1-acid glycoprotein and human plasma albumin. In humans, the steady state plasma concentrations are attained in 10 days, and the maximum elimination $t_{1/2}$ is 48 hours. Gefitinib has a greater tissue distribution and a longer elimination $t_{1/2}$ than erlotinib [25]. Gefitinib is mostly removed from humans through the liver. However, it was noted that the plasma clearance in male rats and dogs was about 25 and 16 mL/min/kg, respectively [24].

Formulation research to improve gefitinib BA

New formulations of gefitinib BA are being studied. Gefitinib has been used in investigational and clinical settings to study its *in vivo* fate as a solid in granules or dissolved/diluted/suspended in various mediums. It is generally accepted that the water solubility, therapeutic effectiveness, and stability of the specific medications impact their oral BA. Numerous research has addressed innovative preparation methods to stabilize and prevent gefitinib from deterioration, upsurge its water solubility to increase BA, sustain release, and target it to particular regions using multi-particulate formulae and colloidal carriers. Table 2 lists these test formulations, their objectives, and their excipients. Table 3 explains the influence of gefitinib formulations on PKs.

Formulations as an alternate treatment for patients unable to swallow tablets

Mireille prepared the granular form of gefitinib to increase exposure in single doses of 250 mg compared to the standard tablet for patients who cannot or will not swallow the standard tablet[60]. There was 1.4-fold intra-subject heterogeneity in AUC and 1.7-fold in C_{max}, which is slightly better than tablets [25], and their study suggested that the granular formulation of gefitinib was exposed similarly to tablets, with good reproducibility in patients [17].

Methods (formulations) to attain targeted and/or sustained release of gefitinib

Several studies have focused on developing formulations with sustained release properties, such as nanometer-scale colloidal carriers or multi-particulate forms, with the ultimate goal of transporting and delivering gefitinib to specific target sites.

Multifunctional Nanoparticles

Researchers George Simon and colleagues. found that gefitinib had good anticancer activity and gave good palliation in a group of patients who had previously had chemotherapy or radiation for advanced NSCLC [26].

Nanoparticle aerosol lipid matrices

The development of nanoparticle aerosol lipid matrices (NALM) allowed for the sustained delivery of gefitinib. A precursor solution of gefitinib and stearic acid, with a weight ratio of 1:4 w/w, was used to synthesize NALM in an aerosol reactor. The organic solvents used for this process had vapor pressures ranging from high to low. The geometric standard deviation ranged from 1.6 to 1.9 nanometers, while the mean mobility diameter fell somewhere between 123 and 132 nanometers. The drug loading of 20% w/w gefitinib in NALMs resulted in faster drug releases, lower degrees of crystallinity, consistent entrapment efficiencies of around 100%, and higher drug loading. Using pH 7.2 for 10 days, these NALMs exhibited a rapid initial release and a long sustained release with variable release rates. Based on their research, the authors determined that NALMs loaded with gefitinib might be used in sustained delivery systems [27].

Gefitinib-loaded Polycaprolactone Microcapsules

A simple solvent evaporation approach was used to make gefitinib-loaded polycaprolactone microcapsules by Roy in 2012. In the aqueous phase, the researchers evaluated three drug-polymer ratios (1:2, 1:4, and 1:6) and three stabilizer/surfactant concentrations (0.25%, 0.50%, and 0.75%). The goal was regulated medication release following weekly subcutaneous insertion for localized targeted therapy. With an average particle size diameter of only 201±3.05 ì and a drug entrapment efficiency (EE) of 90.19±2.61%, these microcapsules were the most effective. The microcapsules' EE and particle size were affected by the surfactant content. Particle size and EE both reduced with increasing surfactant concentration, likely due to increased gefitinib migration into the surfactant solution. As demonstrated by its in-

vitro-releasing property, gefitinib adhered to the Higuchi model and maintained drug release until day seven[28].

Gefitinib incorporated folate decorated bovine serum albumin conjugated carboxy methyl-â-cyclodextrin nanoparticles (FA-BSA-CM-â-CD NPs)

Yijie Shi and colleagues developed FA-BSA-CM-â-CD NPs, which are nanoparticles loaded with Gefitinib and coated with folate. These NPs improve drug delivery to cancer cells. They used carbodiimide coupling to attach BSA to CM-â-CD. Nanoparticles (NPs) had folate (FA) a tiny targeting molecule, attached to their surfaces. Good homogeneity in particle size, negative charge, and monodispersity were observed in these NPs. The average size of the spherical, monodisperse nanoparticles was 90.2 nm, and they had a negative surface charge of -18.6 mV. Their research showed that the produced NPs could significantly improve drug absorption and increase toxicity to folate receptor-positive Hela cells without causing any cytotoxicity. Thus, FA is a potent targeting chemical, and the generated nanoparticles offered a fresh approach to killing cancer cells in humans [29].

Apoferritin (AFt)-based drug delivery system

A nanoscale drug delivery system containing gefitinib encapsulated in human apoferritin (H-Aft) was developed and successfully tested by Anchala 2015, the researchers discovered that the nanocomposite had enhanced antitumor activity and improved drug selectivity for HER2 overexpressing cells. They concluded that H-AFt is an effective vehicle for the localized administration of gefitinib. The developed drug delivery devices demonstrated strong anticancer effects in vitro, sustained drug release, and a dose-dependent reduction of cancer cell proliferation. The therapeutic efficacy, target toxicities, and drug deposition in normal tissue are all improved by the encapsulated substance [30].

Chitosan nanoparticles

Xiwei Yu in 2015 developed a novel nano-delivery method through sustained release, lesser particle size, and great encapsulation efficiency to effectively co-deliver the gene (shMDR1) and anticancer medication (gefitinib) and overcome the multidrug resistance effect. Researchers looked into the gene's transfection efficiency in vitro, serum stability, nuclease protection, and the impact of nanoparticle co-delivery in reversing multidrug resistance. In particular, their findings showed that these NPs might enhance the effectiveness of cancer treatments by targeting resistant cells and thereby overcoming multidrug resistance [31].

Different methods to improve gefitinib aqueous solubility

Complexation with hydroxypropyl-â-CD (HP-â-CD) and randomly methylated-â-cyclodextrin (RM-â-CD)

The formation of stable inclusion complexes with hydroxypropyl-â-cyclodextrin (HP-â-CD) and randomly methylated-â-cyclodextrin (RM-â-CD) in both liquid and solid states was studied by Lee and his batch. to enhance the solubility and dissolution of gefitinib. The researchers demonstrated that this approach could overcome resveratrol's limited water solubility. The drug formed weak complexes with association rate constants (Ks) of 71.4 and 102.5 M"1 for HP-â-CD and RM-â-CD, respectively, in water and an acetate buffer (pH 4.5), according to the phase solubility studies. The stable inclusion complexes had Ks of 458.9 and 1096.2 M"1 for HP-â-CD and RM-â-CD, respectively. They used the freeze-drying method to solidify the complexes, and then they used X-ray diffractometry (X-RD) and differential scanning calorimetry to characterize them. The amorphous-produced inclusion complexes improved gefitinib solubility and dissolution, according to their findings [4].

Gefitinib-loaded nanoparticles

Increased anti-cancer activity on A549 lung carcinoma cells and A431 skin carcinoma cells was demonstrated by gefitinib nanoparticles, according to a plausible mechanism outlined by Jasmine Kaur in 2013. In addition to blocking EGFR on the plasma membrane, their nuclear localization activates p300/CBP, which leads to hyperacetylation of histone H3. They are the first to show that GNPs activate p300/CBP histone acetyltransferases, which in turn enhance cell death [32].

Gefitinib colloidal gold nanoparticle (AuNP)

Aiming for a more effective weapon against lung cancer cells Anh to determine the efficacy of gefitinib colloidal gold nanoparticle (AuNP) conjugates against A549, NCIH460, and NCI-H1975s lung cancer cells, Thu Ngoc Lam

and team. conducted a self-assembly experiment. When applied to lung cancer cells, these AuNPs boost gefitinib's effectiveness. After building the GF-AuNPs-antibody, their research demonstrated that cell viability dropped by 30~80%. According to their findings, the TK inhibitor of GF was effectively conjugated with AuNPs [33].

A Ligand-conjugated pH-sensitive Polymeric Micelles

To explore gefitinib's tumor-targeting potential, Shi-Jiang Wang in 2015 created a pH-sensitive nanosystem using mannose conjugates. They discovered that these complexes considerably raised the percentage of cells in the apoptosis and necrosis region and that these nano micelles had a superior anticancer impact in A549 cancer cells, inhibiting cancer cell proliferation. In comparison to the free drug, the drug-loaded nano micelles exhibited a 5-fold increase in t1/2 and a 7-fold increase in drug accumulation in tumor tissues. This led the scientists to the conclusion that nano micelles may be a viable delivery mechanism for increasing Gefitinib's therapeutic efficacy in lung malignancies [34].

Chitosan nanoparticles

To overcome drug resistance that has developed over time, chitosan nanoparticles (CS NPs) were synthesized by combining chloroquine (CQ) with gefitinib (Gefitinib). Nanoparticles with these characteristics displayed a modest size, a positive zeta potential, biphasic medication release control, increased inhibition rates, and improved cell apoptosis. Combination therapy may be able to boost the efficacy of cancer treatments, particularly in cases of resistance, and overcome acquired resistance, according to the researchers [35].

Gefitinib nanosuspensions

Navya Sree Kola Srinivas, developed gefitinib nanosuspensions by enclosing the drug in Eudragit RL100 and dispersing it in a stabilizer solution, polyvinyl alcohol, and polyvinyl pyrrolidone K30. Their goal was to decrease production costs while improving BA, quality, and safety. Results from in vitro cytotoxicity tests using the Vero cell line showed that the formulation does not cause any harm. Within eighty-four hours, the drug release was six times higher than that of the basic medication. Their findings of a relative BA of nanosuspension that was 1.812 times higher than before validated the suitability of the current formulation for improving the oral BA of gefitinib [36].

Controlled released spray-dried matrix systems

Controlled released spray-dried matrix systems were created by Chandraiah Godugu, using spray-drying technology to increase the solubility of anti-cancer medications that are poorly water-soluble. They made Gefitinib spray-dried (Gef-SD) formulations using a spray cannon that allows two liquid systems to flow through a single nozzle. Results showed that the microparticles enhanced anti-cancer activity by a factor of 1.75, BA by a factor of 9.14, tumor size by a factor of 1.48, and volume by a factor of 1.75, according to their in vitro drug release and Caco-2 penetrability studies. A431 xenograft tumor models also showed an increase in PD effects when these formulations were used. According to the findings, the Gef-SD formulations significantly enhanced the therapeutic efficacy of Gefitinib [37].

Gefitinib polymeric microspheres

Using an oil-in-water emulsification technique, Weiluan Chen in 2017 created polymeric microspheres filled with drugs. To determine if the particle size impacts the drug loading efficiency and release profile, their primary objective was to examine the characteristics of drug-loaded microspheres with varying fractions of size (5 ± 1, 32 ± 4, 70 ± 3, and 130 ± 7 ìm). The results showed that gefitinib was amorphously distributed throughout the PLGA matrix and that the Tg of PLGA did not change, suggesting that tiny drug particles had formed inside the matrix. Drugs were released rapidly from microspheres smaller than 50 ìm through diffusion, while bigger microspheres exhibited a sigmoidal drug release pattern with early diffusion and later erosion. The results demonstrated that larger microspheres deteriorated at a quicker rate than their smaller counterparts. They found that the size of the microspheres was crucial for getting well-defined and reproducible sustained release depots [38].

Poly (L-lactic acid) (PLLA) microspheres

Qing Lin, recently used supercritical anti-solvent (SAS) technology to encapsulate gefitinib into poly (L-lactic acid) (PLLA), resulting in the creation of PLLA microspheres. The findings demonstrated that the particles were round, with a smaller and narrower particle size, a maximum drug loading capacity of 15.82%,

and an entrapment effectiveness of 94.91%. The particle size was also lower (D50 = 2.48 ìm). These microspheres improve gefitinib's efficacy, stability, and safety, according to the authors [39].

â-cyclodextrin-mediated single bimolecular inclusion complex

To decrease its cost and boost its action, Souvik Basak, effectively produced a multimolecular inclusion complex employing the cosolvent evaporation approach using â-cyclodextrin (â-CD) as a carrier. According to the characterization investigations, the inclusion complex creation of the two medicines with â-CD was successful, and the resulting drug release was ten times better and followed zero-order kinetics. The results showed that creating inclusion complexes with numerous molecules in one cavity is a potential way to increase the dosage form's effectiveness [1].

Silver nanoparticles (AgNPs)

Samra Khalid, extracted green synthesized silver nanoparticles (AgNPs) from the Aerva javanica plant, and gefitinib was conjugated to these AgNps. The prepared nanoparticles were evaluated by Ultraviolet (UV), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM), and found that those were spherical with an average size of 5.7nm, and in a shorter period, the drug was delivered effectively in tumor cells. They also analyzed the cell viability in the human breast cancer cell line (MCF-7) and found that drug-loaded silver NPs were 50% more effective than gefitinib alone and there was a reduction in toxicity [40].

Gefitinib-loaded nanoparticles

Problems like organ toxicity, treatment resistance, and disease relapse can be avoided by using a solid dispersion approach to generate GEF-NPs, which are nanoparticles loaded with poly (e-caprolactone)-poly(ethylene glycol)-poly(e-caprolactone) (PCEC). The produced nanoparticles were seen to be spherical, and monodispersed, with a tiny particle size (less than 24 nm), a zeta potential of -18 mV, a loading percentage of more than 92%, and an encapsulation percentage of more than 9%. Additionally, the medication was discovered to be slowly released during the investigation. They improved anticancer efficacy, decreased adverse effects, and greatly increased in vivo survival time. When tested on the A549 cell line, the nanoparticles demonstrated potent cytotoxicity that was both dose- and time-dependent. The results of the research indicated that these nanoparticles might be a novel non-small cell lung cancer treatment [41].

Modified Gefitinib Conjugated Fe3O4 nanoparticles

Suresh Thangudu, successfully synthesized a modified GEF (mGEF) drug and conjugated to Iron oxide nanoparticles (Fe3O4 NPs) for the treatment of NSCLC via magnetic resonance (MR) image-guided drug delivery. A traditional EDC coupling pathway uses mGEF to directly conjugate to Fe3O4 NPs to overcome the drug leakage issues. As a result, they found in vitro drug delivery on mGEF- Fe3O4 NPs exhibits excellent anticancer effects towards the PC9 cells selectively, with an estimated IC 50 value of 2.0 ìM. Additionally, in vivo MRI and PET results demonstrate that the NPs could accumulate in tumor-specific regions with localized cell growth inhibition. Their results also revealed that outer tumor region exhibiting a stronger contrast than the tinner tumor region which may due necrosis in inner tumor region. In vivo biodistribution further confirms Fe3O4 NPs are more biocompatible and are excreated after the treatment. Overall, we believe that this current strategy of drug modification combined with chemical conjugation on magnetic NPs will lead to improved cancer chemotherapy as well as understanding the tumor microenvironments for better therapeutic outcomes. In vivo MRI and PET data show that the NPs aggregate in tumor-specific areas and impede cell proliferation. The outer tumour region had a sharper contrast than the tinner tumour region, possibly due to necrosis in the interior region. In vivo biodistribution reveals that Fe3O4 NPs are more biocompatible and produced following treatment. Their present technique of drug modification and chemical conjugation on magnetic nanoparticles aims to enhance cancer chemotherapy and better understand tumour microenvironments for better therapeutic results [42].

Gefitinib incorporated in nanoliposomes

To study drug accumulation in tissues and cells, and to characterize drug fluorescence as a tool for formulation development, Brian Trummer in 2012 created a liposome formulation with a high payload of the EGFR inhibitor gefitinib. The environmental polarity determined the peak

excitation and emission wavelengths, which ranged from 385 to 465 nanometers. Water emitted almost no light, but in nonpolar solvents, on membranes, or when bound to serum proteins, the light was very bright. To indicate that reaching therapeutic concentrations in humans is feasible, they included a large amount of medication per mole of phospholipid carrier. Researchers found that stable liposomes made of distearoyl phosphatidylcholine, polyethylene glycol, distereoyl phosphatidyl ethanolamine, and cholesterol (9:1:5 mol:mol:mol) contained 40-60 mol% of gefitinib. Because the medicine had greater tumor-skin and tumor-bone marrow deposition ratios, it was able to lower the toxicity level[43]. Maram , encapsulated MBZ, a new and promising Ran GTPase inhibitor, in conventional nanoliposomes to improve gefitinib's anticancer cytotoxic effect on A549 and resensitize its performance. One of the initial treatments for lung cancer is gefitinib. After synthesizing the nanoliposomes by the thin film hydration extrusion process, their average size, Poly Dispersity Index (PDI), and zeta potential were measured and produced in an accurate nanosized and very stable formula after a month of storage at 4°C[44]. The cellular experiment demonstrated that, after 72 hours, the produced drug-laden nanoliposomes were more effective and synergistic than the free drug precursors against A549 cell lines. The nanoliposomes containing the two

Table 1. The Pharmacokinetic (PK) key attributes of gefitinib[15]

M.W	447 Da
C_{max}	3–7 hours after oral dosing
Oral BA	60%
Terminal $t_{1/2}$	48 hours
Time to reach a steady state	7–10 doses
Protein binding	90%
Enzymatic metabolism	CYP3A4
Main metabolite	O-desmethyl-gefitinib
Excretion	Feces 86% and urine (4%)

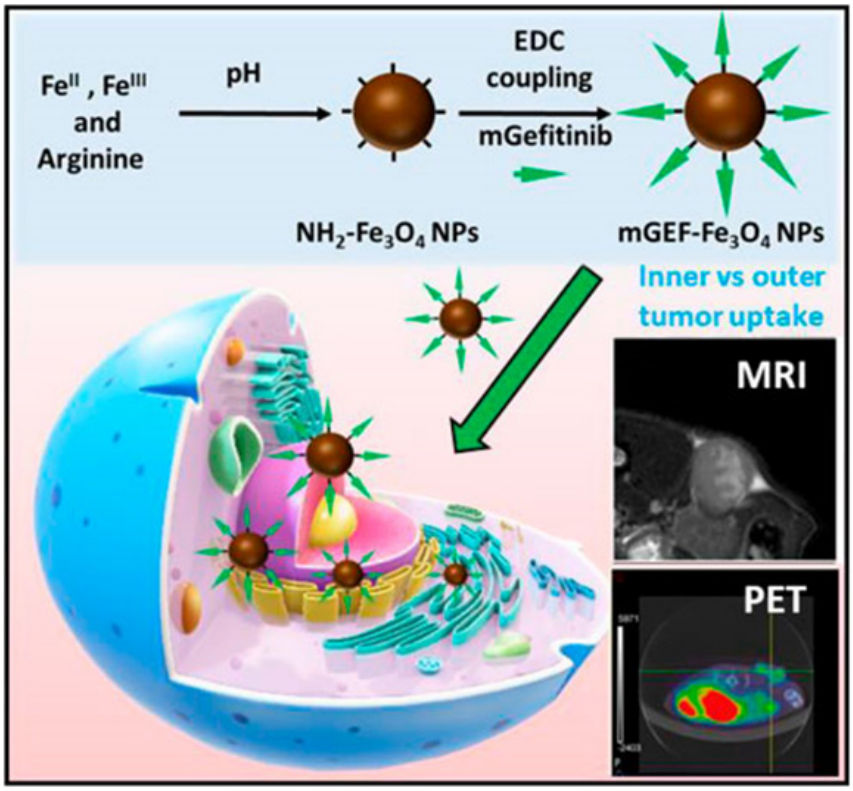

Fig. 1. Schematic illustration of chemically conjugated modified gefitinib drug to Fe3O4 NPs for image-guided chemo delivery for NSCLC treatment [42].

drug combinations were the most effective. When compared to the free medications, the liposomal formulation performed better in the wound closure test and the colony experiment [45].

One major obstacle to treating lung cancer is the emergence of resistance to gefitinib. In 2023, Puwei Song finished researching the underlying mechanism associated with gefitinib resistance and validating the corresponding experiments in lung cancer [46]. The goal of this study is to better understand how lung cancer patients develop resistance to gefitinib. Data on the gene expression patterns of both control and gefitinib-resistant cells were collected. When the data from the TCGA and GDSC databases were integrated, six genes were shown to be implicated in gefitinib resistance at the cell and tissue levels: RNF150, FAT3, ANKRD33, AFF3, CDH2, and BEX1. Additionally, they found that fibroblasts in the NSCLC microenvironment expressed the majority of these genes. Consequently, they meticulously investigated fibroblast's role in the NSCLC microenvironment, including its biological effects and cell interactions. Because of its association with prognosis, CDH2 was ultimately selected for further study. In vitro experiments confirmed the importance of CDH2 in NSCLC for cancer promotion. Furthermore, cell viability studies showed that blocking CDH2 significantly lowers the IC50 of gefitinib in NSCLC cells. According to GSEA, CDH2 may significantly affect the activation of the PI3K/AKT/mTOR signaling pathway [47].

_____Hossein Alizadeh, utilised a microfluidic chip to synthesise a nanocarrier for the anticancer medication geftinib. The nanocarrier was made using natural polymers such as chitosan and alginate. Secondary amine functional groups interact during the nanocarrier production. Gefitinib molecules are combined with carboxylate functional groups of alginate polymer to produce a main nucleus. The nanocarrier is then formed by self-assembly of chitosan and alginate polymers on a microfluidic chip. The chip was created using laser etching poly(methyl methacrylate) polymer sheets. The nanocarrier was characterised using

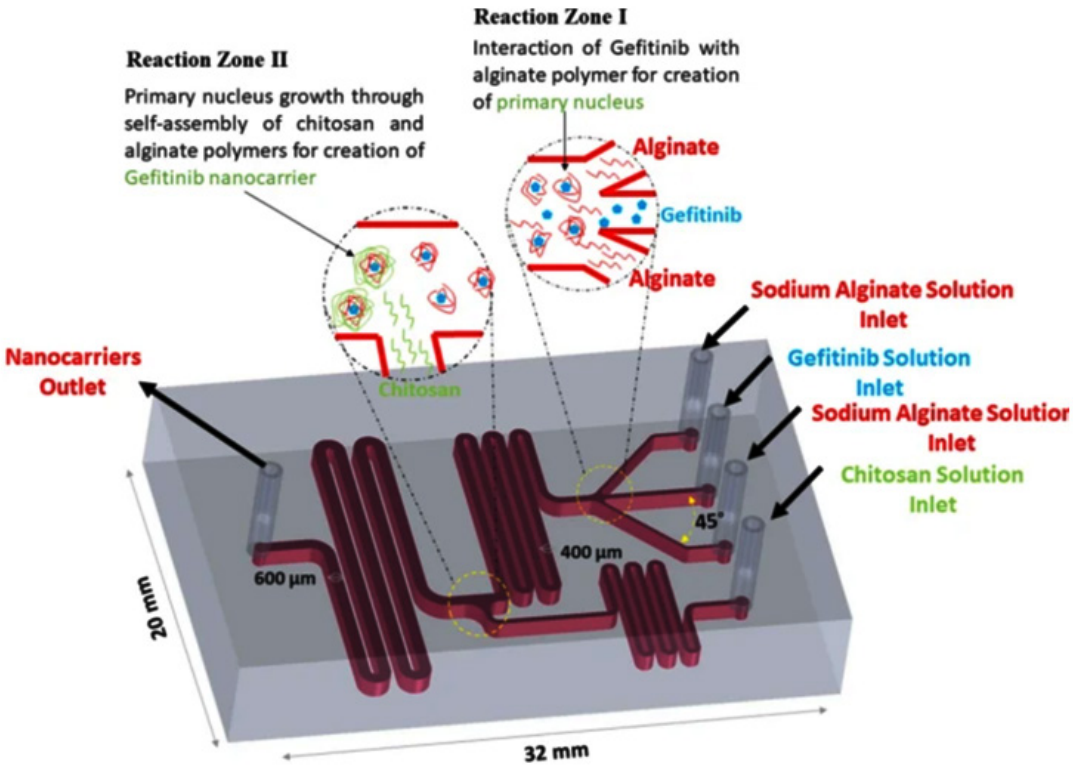

Fig. 2. 3D illustration of designed microfluidic chip for the synthesis of gefitinib nanocarrier[48]

Table 2. Different formulations of gefitinib along with their objectives and excipients

Objective	Pharmaceutical form	Excipients
The alternate course of treatment for those who prefer not to or are unable to take the regular pill form.	Granular formulation [25].	Gefitinib tablet.
Molecular targeted cancer therapy of gefitinib.	Multifunctional Nanoparticles [26,58].	Review.
Sustain gefitinib release, enhance drug loading, and control the crystallinity.	Nanoparticle aerosol lipid matrices [27].	Stearic acid, di chloro methane, ethyl acetate, and chloroform.
Control and target gefitinib release.	Polycaprolactone Microcapsules [28].	Polycaprolactone, Polyvinyl alcohol, chloroform, Potassium dihydrogen phosphate, Disodium hydrogen phosphate, and Sodium lauryl sulfate.
Improve the solubility and stability of the medicine, make it more toxic to folate receptor-positive Hela cells, and increase its efficacy in targeting tumors.	carboxy methyl-²-cyclodextrin nanoparticles coated with folate and bound to bovine serum albumin [29].	Bovine serum albumin, Carboxymethyl-²-Cyclodextrin Sodium Salt, 1-ethyl- 3-(3-dimethyl aminopropyl) carbodiimide (EDAC), and folic acid.
Improve drug selectivity for HER2 overexpressing cells, enhancing therapeutic efficacy and antitumor activity, sustaining and targeting gefitinib delivery.	Apoferritin (AFt)-based Drug Delivery System [30].	DMSO, heavy chain apoferritin (H-AFt).
Sustain gefitinib release, improve encapsulation efficiency, improve cancer treatment efficacy, reduce particle size, and overcome multidrug resistance.	chitosan . nanoparticles [31]	3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), chitosan, proteinase K, pGCsi-U6/Neo/GFP-shRNA-expressing pDNA (pEGFP), pGCsi-U6/Neo/GFP-MDR1-shRNA-expressing pDNA (shMDR1) and Gefitinib-resistant Hela cells.
Increase solubility and dissolution.	Cyclodextrin inclusion complexes [4].	Glacial acetic acid, PVP K30, HP-²-CD , RM-²-CD and HPMC E3.
Minimize adverse effects while simultaneously increasing tumor deposition, decreasing free drug concentrations, and decreasing drug deposition in healthy tissues.	Nano liposomes [32].	Distearoyl phosphatidylcholine, poly ethylene glycol distereoyl phosphatidyl ethanolamine, cholesterol, polycarbonate filters, and HPLC grade solvents.
Enhance cancer activity.	Nano particles [33].	PLGA, PVA and Chloroform.
Increase potency against lung cancer cells.	Colloidal gold particle conjugates [34].	HAuCl4, citrate, Dimethyl sulfoxide (DMSO), and The (ortho-pyridyl) disulfide - poly(ethylene glycol) - N hydroxyl succinimidyl ester (OPSS-PEG-NHS) linker.
Improve the medicine's release, make it more	A pH-sensitive Polymeric Micelle	Mannose, PLGA, PEG, Poly histidine.

effective against cancer in A549 cells, and lengthen the half-life of the drug.	Complex Conjugated with a Ligand [35].	
To enhance the effectiveness of cancer treatments and overcome acquired drug resistance.	chitosan nanoparticles [36].	Chitosan, acetic acid and sodium Tripolyphosphate.
Improve gefitinib BA, quality, and safety, and reduce manufacturing costs.	Nano suspension [37].	Eudragit RL100, Polyvinyl alcohol, Polyvinylpyrrolidone (PVP) K30, Methanol, Dimethyl sulfoxide, Dulbecco's Modified Eagle's Media, and trypsin ethylenediaminetetraacetic acid, Fetal bovine serum (FBS), sterile phosphate-buffered saline (PBS), 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT), and antibiotic solution.
Improve oral absorption, and enhance PK and PD activity.	A spray-dried matrix technology with controlled release [38].	HPMC E3 grade, chitosan, Hydroxypropyl ²-cyclodextrin (HP²-CD), succinic acid, Vitamin E-TPGS, antibodies Ki67, Cyclin D1, p53, survivin, cleaved caspase- 3, (vascular endothelial growth factor) VEGF, vimentin, VEGF ELISA kit, Caco-2 cells, human epidermoid A431 cells, Fetal bovine serum (FBS), Trypsin–EDTA, antibiotic-antimycotic solutions, HBSS and HEPES buffer.
To know the properties of different-size fractions.	Polymeric (PLGA) Microspheres [39].	PLGA 5004A, PVA, disodium hydrogen phosphate, sodium azide, Phosphate buffered saline, dichloromethane, tetrahydrofuran, acetonitrile, Dextran, glycidyl methacrylate, ammonium peroxydisulfate, N, N, N', N'-tetra methyl ethylene diamine, 4-(N, N-dimethylamino) pyridine and Dialysis tubes.
Improve safety, stability, and potency.	of poly (L-lactic acid) microspheres[40].	CO2 (> 99.9% wt), poly (L-lactic acid), tween 80, dichloro methane, ethanol, and phosphate buffer saline.
Increase gefitinib drug release and efficiency.	the b-cyclodextrin-mediated single bimolecular inclusion complex [1].	Beta-cyclodextrin, ethanol, acetonitrile, methanol, and Zinc sulfate (ZnSO4).
Improve gefitinib efficiency and reduce toxicity.	Silver Nanoparticles [41].	Aerva javanica plant extract, $AgNO_3$, polyethylene glycol, ethanol.
Improving anticancer efficacy boosts steadiness, lessens adverse effects, and drastically extends longevity.	Nanoparticles [42-46].	Poly (ethylene glycol), e-caprolactone, stannous octoate, RPMI- 1640 3 -(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide, Dimethylsulfoxide, anhydrous ethanol, methanol isopropyl alcohol, IL-6 ELISA Kit, TGF-b1 ELISA, Hydroxyproline assay kit, Ki-67, EGFR, and CD31 polyclonal antibody.

Table 3. Influence of formulation on gefitinib PKs

Formulation	Dose of gefitinib and route of administration	Experimental model	PK characteristics (plasma appearance/ T_{max}/C_{max})
β cyclodextrin inclusion complex.	The oral dosage is 86.78 mg/kg of Gefitinib Simvastatin inclusion complex (GSBCD), which contains 25.65 mg of Gefitinib and 20.82 mg of Simvastatin.	9 healthy Albino rats.	Enhanced plasma retention and a tenfold increase in medication release[1,4,29].
Granular formulation.	250 mg, oral route.	18 healthy male subjects.	AUC and C_{max} intra-subject variability of 1.4- and 1.7-fold, respectively, which is somewhat better than that of tablets[17].
Oral dose of gefitinib.	250 mg gefitinib tablet.	12 male healthy subjects.	The AUC was 2162±81 ng/mL compared to 4996±64 ng/mL and the mean plasma C_{max} was 99.2 ng/mL, as reported in reference[18].
Oral dose of gefitinib.	administration of 50 and 150 mg/kg in one go.	Mice model.	The AUC(0-24 h) differs by 1.5 times[19].
Oral dose of gefitinib.	The Doses of 50, 100, and 200 mg/kg.	Mouse model.	With a $AUC_{total\ brain}/AUC_{total\ blood}$ of 0.4 in the 50 mg/kg group and 0.7 in the 200 mg/kg group, AUC_{CSF}/AUC_{free} blood of 0.21 and0.18, respectively, were reported in the aforementioned study[20].
Oral dose of gefitinib.	250 mg gefitinib.	72-year male subject.	It appears that CAPD had minimal impact on the PKs of gefitinib[21] since the plasma concentration hit the steady state level by day 16, and by day 46, it was 538.4 ng/ml, with a peritoneal dialysis fluid concentration of 34.6 ng/ml.
Oral dose of gefitinib.	250 mg gefitinib.	Primary cell lines from non-small cell lung cancer in humans.	Without activating mutations, the rate at which lung cancer cells metabolize gefitinib in response to CYP1A1 activity may serve as an early predictor of the NSCLC cells' receptivity to gefitinib[22].
Oral dose of gefitinib.	250 mg of gefitinib.	Fifteen individuals were diagnosed with lung cancer.	Patients who experienced a partial response (PR) to gefitinib had a considerably lower Cmax than those who had stable illness[23].
IV dose.	Intravenous dosing (5 mg kg).	Rat and Dog.	In rats and dogs, the plasma $t_{1/2}$ of gefitinib was 3-6 hours, though studies utilizing a more accurate HPLC-MS assay reported higher $t_{1/2}$ estimations (7-14 hours)[24].

It is controlled released spray-dried matrix system.	50 mg/kg, oral route.	Sprague Dawley rats.	A 9.14-fold increase in the AUC[38].
Nanostructured lipid carriers.	50 mf of gefitinib of nano lipid carrier.	HCT-116 cells.	When compared to Gefitinib alone (IC_{50} = 20.88 μM), the cytotoxicity of optimized nanogefitinib increased by 4.5 times (IC50= 4.642 μM)[59].

FT-IR, DLS, SEM, and TEM techniques. The synthesised nanocarrier had a size distribution of 5.30± 2.60 nm and achieved 68.4% encapsulation efficiency under optimal circumstances. The loading efficiency was calculated to be 50.2 mgg"1 of nanocarrier. Drug release tests indicate that the nanocarrier is pH sensitive, releasing more gefitinib in acidic environment. Cytotoxicity of synthesised nanocarriers. The study on A549 non-small cell lung cancer found that the synthesised nanocarrier had a reduced IC50 value compared to the free medication. Cytotoxicity tests indicate that the materials utilised for nanocarrier manufacturing are not significantly harmful. The microfluidic-assisted approach has advantages over earlier methods, including faster synthesis, comparable encapsulation efficiency, and loading capacity [48]

Cellular photo (geno) toxicity of gefitinib after biotransformation

Three chemically reactive metabolites with higher UV light absorption—O-Demethyl Gefitinib (DMT-GFT), 4-Defluoro-4-hydroxy Gefitinib (DF-GFT), and O-Demorpholinopropyl Gefitinib (DMOR-GFT)—are produced when gefitinib is bioactivated mainly through Phase I hepatic metabolism. To better understand how photosensitivity diseases could be caused, Meryem El Ouardi and colleagues looked into the possibility that gefitinib metabolites could be involved. Their research shows that gefitinib's biotransformation causes cellular photo(geno)toxicity, which is a double-edged sword. This information is essential for the growth of new TKIs that are being developed to foresee and reduce potential phototoxic adverse effects. When prescribing TKIs to cancer patients, doctors must take into account all of these factors to set the conditions of use and suggest photoprotection recommendations [49]. Ultimately, a computed tomography-guided percutaneous lung biopsy revealed an invasive mucinous adenocarcinoma of the lung in a 56-year-old Chinese man who had been initially diagnosed with severe pneumonia and had a smoking history and epidermal growth factor receptor mutation-positivity. Radiographic signs of bilateral lung consolidation showed significant improvement following 30 days of beginning gefitinib treatment, and bronchorrhea and dyspnea improved within 24 hours. Treatment has been going well for over 11 months with no signs of recurrence or any side effects. According to Guo-Chun Ou and colleagues, gefitinib is an important tool for the detection and management of lung-invasive mucinous adenocarcinoma (LIMA) [50].

To control triple-negative breast cancer (TNBC), another therapeutic strategy is to target the EGFR. Gefitinib, an EGFR tyrosine kinase inhibitor, was specifically delivered to TNBCs using EGFR-targeting micelles created by Won, Gef was released from the EGFR-targetable micelles in a continuous way as well as self-assembled nano-sized structures. Additionally, they more effectively treat TNBC cells than TNBC cells with low or negative EGFR expression. They inject Gef into TNBC cells precisely [51]. For patients with advanced NSCLC who test positive for the EGFR gene, researchers performed A Retrospective Exploratory Study to assess the efficacy and safety of gefitinib + anlotinib. For patients with EGFR-positive NSCLC who had not been treated before, this trial provided solid evidence that a gefitinib + anlotinib combo demonstrated good efficacy and a tolerable safety profile. Prospective clinical studies with follow-up participants are also necessary to confirm the findings [52]. The initial results on the safety, tolerability, and survival rates of patients treated with gefitinib for NSCLC were reported by Hirsch and his colleagues. Some patients in the IRESSA Clinical Access Programme (ICAP) were able to respond to gefitinib for an extended period; this group may not represent the overall population of patients who survive three years, but over 60%

of patients in the program were able to do so after fifteen years. Treatment with gefitinib lasted more than ten years on average, and patients had few side effects. To fully comprehend the genetic and clinical factors associated with such a lasting impact, additional study is required [53].

Scientists from Saudi Arabia have optimized Gefitinib-Loaded Nanostructured Lipid Carriers (NLC), which were previously utilized to delay medication release and have inherent lymphatic tropism. A GEF-NLC, a system for the lymphatic delivery of drugs with a low rate of release, was their intended means of accomplishing this. With the help of experimental design, a stable GEF-NLC was developed to treat metastatic lung cancer by delivering drugs via lymphatics. To determine the optimal formulation, researchers examined the GEF-NLC formulations' in vitro drug release. Using the MTT assay, we determined whether GEF-NLC was cytotoxic to free GEF. Positive physicochemical properties were associated with the enhanced GEF-NLC formulation, which achieved an EE of over 90%. It is worth mentioning that the formulation that was made only released 57% of the medicine after 24 hours, all while keeping GEF. The optimized formulation offers an approach to improve GEF's therapeutic effectiveness in managing metastatic lung cancer with reduced systemic toxicity. Experimental evidence suggests that GEF-NLC LCFA alters GEF's cytotoxic effect on A549 cells. Based on all of the data, they concluded that NLC is a great way to make GEF work better against lung cancer. With fewer side effects, GEF-NLC(LCFA) offers new possibilities for research to improve GEF's therapeutic profile for lung cancer treatment [56]. Hongmei, compare gefitinib to other commonly used drugs in different therapeutic situations, looking at its efficacy, toxicity, and EGFR mutation status. Nineteen RCTs with 6,554 patients with NSCLC were pooled for this random-effect meta-analysis. They found that Gefitinib, in individuals with EGFR mutations, will have a lower Objective Response Rate (ORR) than chemotherapy but a higher one than other targeted medicines. Gefitinib can lessen the chances of hematologic toxicity as compared to the control group. To confirm these results, more study is needed, especially studies including EGFR-mutant patients [55].

Scientists recently performed a meta-analysis to compare the safety and effectiveness of gefitinib plus chemotherapy to gefitinib alone in patients with non-small cell lung cancer[56]. Clinical trials comparing gefitinib with chemotherapy against gefitinib alone were searched for in the databases. The odds ratios (ORs) for progression-free survival (PFS), overall survival (OS), disease control rate (DCR), complication grade 3 (OR), and other outcomes were calculated by pooling raw data from the trials that were included in the analysis. A total of 1,528 individuals with NSCLC were included in the study, which was carried out on ten separate investigations. A combination of gefitinib with chemotherapy considerably enhanced overall response rate (ORR), disease-free survival (DFS), progression-free survival (PFS), and overall survival (OS) compared to gefitinib alone. Consistent results were observed in the subset whose members had a positive EGFR mutation. When gefitinib and chemotherapy were administered together, the odds of complications (e"Grade 3) were 3.29 times higher. Combining gefitinib with chemotherapy may improve disease response and survival rates in individuals with advanced NSCLC, according to their research [57]

Future scope for gefitinib delivery systems

The novel medicine gefitinib has shown anticancer effectiveness in patients with advanced, relapsed non-small cell lung cancer. Clinical trials are investigating the use of gefitinib in a variety of other common solid tumor forms than NSCLC. Phase I clinical trials demonstrated that gefitinib was well-tolerated, and clinical action was shown at doses significantly lower than the maximum tolerated. It is common for gefitinib to have mild to moderate side effects, although these are usually manageable and even reversible with the correct care.

CONCLUSION

Research efforts have been concentrated on developing novel medicines that target tumor tissue because conventional cytotoxic anticancer therapies can not distinguish between tumor and host cells. Gefitinib blocks signal pathways that solid tumors use to develop and spread; it is an orally active EGFR tyrosine kinase inhibitor. Patients with advanced NSCLC who had already had chemotherapy responded well to gefitinib

250 mg daily in phase II studies. In patients with activating EGFR mutations or in clinically chosen patients (e.g., Asian patients or never-smokers), who are more likely to have these mutations, gefitinib considerably improved progression-free survival (PFS) whether it was given as first- or second-line treatment. Additionally, it enhanced these patients' health-related quality of life metrics and objective response rates.

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Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

Sobitha Rani – Data collection and idea behind the article; Singireddy Anand Reddy - Data collection and idea behind the article; Pola Kranthi kumar – Drafting and communicating; Anusha Kusuma – Proof reading; Pulla Udaya Chandrika – proof reading

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