Evaluation of Nose-to-Brain Transport Efficiency of Donepezil Hydrochloride Using In-Vivo Imaging Techniques for Novel Intranasal Alzheimer Drug Formulations

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ABSTRACT

Alzheimer disease (AD) is among the most prevalent causes of worldwide dementia, which is marked by the accumulation of amyloid-β (Aβ) and tau hyper phosphorylation, and the rise of neuron loss. The limitation on the development of therapeutics that can effectively reach the central nervous system (CNS) is due to the restrictive barrier of the blood-brain (BBB). Nose-to-brain (N2B) delivery is a non-invasive method that utilizes a direct pathway of brain delivery by the use of olfactory and trigeminal neural routes. Recent developments in in-vivo imaging, such as those based on positron emission tomography (PET), single-photon emission computed tomography (SPECT), near-infrared fluorescence (NIRF), and magnetic resonance imaging (MRI) have transformed the assessment of drug bio distribution, and real-time monitoring of intranasal transport kinetics are now available. In this review, integration of nanotechnology and imaging modalities are discussed to determine the efficiency of transport and design an effective formulation applicable in the treatment of Alzheimer. Quantitative evidence of increased brain uptake and extended retention after intranasal delivery has been demonstrated to be offered by studies using fluorescent and radiolabelled Nano carriers. Imaging-guided formulation assessment offers important translational data concerning dose optimization, targeting efficiency, as well as safety. Together, these developments underscore the opportunities of the imaging-based approaches to fasten the clinical translation of intranasal therapeutic interventions in AD.

Keywords: Alzheimer's disease; Nose-to-brain delivery; In-vivo imaging; PET; SPECT; Nanoparticles; Intranasal formulations; Blood–brain barrier; Fluorescent probes; Drug targeting efficiency

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1. Introduction

Alzheimer disease (AD) is a neurodegenerative progressive condition impacting over 55 million individuals worldwide, which contributes to 60 70% of the cases of dementia and has been a growing socio-economic burden (Winblad et al., 2016). AD pathologically consists of extracellular amyloid- β deposition, intracellular hyper phosphorylated tau protein deposition, oxidative stress, synaptic impairment, and neuro inflammation (Querfurth and LaFerla, 2010). However, effective treatment solutions are only available in a limited number despite the vast research because most drugs are not able to enter the blood-brain barrier (BBB), a highly selective interface that blocks the movement of therapeutic agents to the CNS (Pardridge, 2020).

The conventional administration routes, such as oral and intravenous delivery, do not reach therapeutic concentrations in the brain because of their enzymatic degradation, first pass in the liver, and low BBB permeability (Abbott et al., 2018). Thus, the nose-to-brain delivery has become a risk-taking non-invasive intervention that directly delivers therapeutic agents to the brain by taking advantage of the olfactory and trigeminal neural pathways (Crowe et al., 2018). This path avoids the systemic circulation and reduces the peripheral side effects and provides a direct route between the nasal cavity and areas of the brain involved in the pathology of Alzheimer disease including the hippocampus and the cortex (Lochhead and Thorne, 2012).

The combination of the sophisticated in-vivo imaging methods has reshaped the evaluation of the N2B drug delivery, offering the quantitative and spatiotemporal picture of the drug biodistribution. Researchers can trace labeled nanocarriers in real-time with the help of imaging modalities like positron emission tomography (PET), single-photon emission computed tomography (SPECT), near-infrared fluorescence (NIRF), and magnetic resonance imaging (MRI), which can be used to determine transport efficiency and kinetics (Calias et al., 2021). This kind of imaging assessment does not only aid the comprehension of the formulation behavior but also aids in the logical development of optimized intranasal nanocarriers to neurodegenerative disorders (Dhuria et al., 2010).

Over the previous decade, the field of nanotechnology has given rise to the emergence of different types of carriers, such as polymeric nanoparticles, liposomes, dendrimers, and nanoemulsions that increase the solubility and stability of drugs and their ability to penetrate the mucosa of the nose (Battaglia et al., 2020). In combination with imaging tracers (radioisotopes or fluorescent dyes), these nanocarriers can be imaged and their distribution and clearance in animal models studied to provide critical pharmacokinetic information. As an example, nanoparticles labelled with 99mTc or 68Ga have been shown to accumulate in the olfactory bulb and the cortex after intranasal administration rather than systemic (Chatterjee et al., 2019).

The current review will evaluate current developments along imaging-guided measurement of nose-to-brain transport of novel intranasal drug formulations in Alzheimer disease. It focuses on the use of multimodal imaging techniques to determine the efficiency of delivery, evaluate the stability of formulation, and translate with clinical development to give evidence.

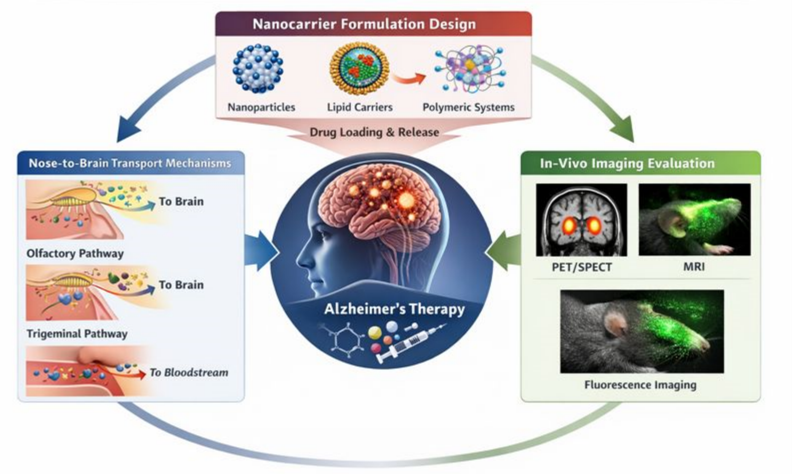

Figure 1: Overview of the study framework showing integration of nose-to-brain transport mechanisms, nanocarrier formulation design, and in-vivo imaging evaluation for Alzheimer's therapy.

2. Pathophysiology of Alzheimer's Disease and Rationale for Imaging-Based Evaluation

Alzheimer disease (AD) is a multifactorial neurodegenerative disease that attacks the hippocampus, neocortex, and limbic system and causes declining memory, learning, and executive functions (Lane et al., 2018). The fundamental pathologic processes of AD are extracellular amyloid- β (Aβ) plaque and intracellular neurofibrillary tangles (NFTs) which are composed of hyper phosphorylated tau protein (Long and Holtzman, 2019). These pathogenic proteins impair neuronal communication, cause mitochondrial dysfunction, and cause chronic neuro inflammatory processes that involve activated microglia and astrocytes (Heneka et al., 2015). In the long run, the result of these processes is a massive synaptic degeneration, loss of neurons, and atrophy of the cortex, which can be observed through neuroimaging.

In neuropathological terms, imaging biomarkers are a non-invasive type of window looking in the disease progression, thus, essential in both the diagnosis and treatment assessment procedures. PET and magnetic resonance imaging (MRI) have played a critical role in the imaging of hallmark lesions of AD in vivo. One of the first imaging agents to identify A8 deposits in live animals was the amyloid-binding tracer [^{11}C]PiB (Pittsburgh compound B) and then fluorine-18 labeled agents [^{18}F]florbetaben, [^{18}F]flutemetamol, and [^{18}F]florbetapir (Villemagne et al., 2018). On the same note, the aggregation of tau is visualizable with PET tracers such as [^{18}F]flortaucipir (AV-1451), and this enables the correlation between the burden of neurofibrillary tangles and cognitive impairment (Leuzy et al., 2020). The MRI architecture identifies the atrophy of hippocampal, enlargement of the ventricles, and cortical thinning, which are used as a surrogate indicator of neurodegeneration (Jack et al., 2018).

The concept of nose-to-brain delivery also implies that the pathology of the AD is an essential topic to discuss since the loss of neurons, disturbed cases of cerebrospinal fluid (CSF), and impaired neurovascular integrity may affect the distribution and clearance of intranasally delivered therapeutics (Sweeney et al., 2019). As an illustration, endothelial dysfunction caused by Aβ is a condition which impairs the BBB permeability and cerebrovascular clearance pathways, and hence it alters the pharmacokinetic of CNS-targeted drugs (Zlokovic, 2011). In addition, in more progressive AD, glial activation, and extracellular matrix remodeling may influence drug diffusion and transport of nanoparticle through the parenchyma (Busche & Hyman, 2020).

The reason behind the inclusion of in-vivo imaging in N2B studies is based on the fact that it is desirable to visualize and quantify the efficiency of drug delivery in the diseased microenvironment of the brain. Traditional bio distribution research based on ex-vivo tissue dissection or bioassays only does not measure dynamic events like absorption kinetics, real-time clearance, and specificity to the region of interest (Yadav et al., 2021). By contrast, the imaging techniques of SPECT, PET, and near-infrared fluorescence (NIRF) provide the high spatial and temporal resolutions of the drug accumulation across brain regions after intranasal delivery. Such techniques can additionally distinguish systemic spillover and direct transport of neurons and confirm the mechanistic nature of N2B pathways (Dhuri et al., 2020).

Recently, labeled Nano carriers have been utilized in brain targeting efficacy visualization and measurement after intranasal injection. As an example, rivastigmine-loaded nanoparticles labeled with 99mTc exhibited much higher uptake rates in the olfactory bulb and cortex, which was evidence of direct uptake of the drug by the N2B pathway, in comparison to intravenous injection (Misra et al., 2018). Similarly, the NIRF imaging of coumarin-6 or DiR-loaded nanoparticles can be combined with real-time monitoring of the progression of formulations in the nasal mucosa and brain in the animal models (Zhu et al., 2019). These optical imaging methods are complementary to radionuclide imaging in that they provide high resolution in mapping surfaces and the cortex, but with poor penetration.

Additional clinical imaging is in-vivo imaging, which can be used to measure pharmacokinetic characteristics, including brain-to-blood ratios, clearance half-lives, and area under the concentration -time curve (AUC). These data when combined with mathematical modeling can introduce a quantitative framework to assess and optimize intranasal drug formulations (Vogt et al., 2020). Moreover, bio distribution, blood flow, and tissue integrity may be evaluated at the same time using multimodal imaging, which is a combination of PET and MRI, and offers a comprehensive view of drug transport in the AD brain (Mishra et al., 2021).

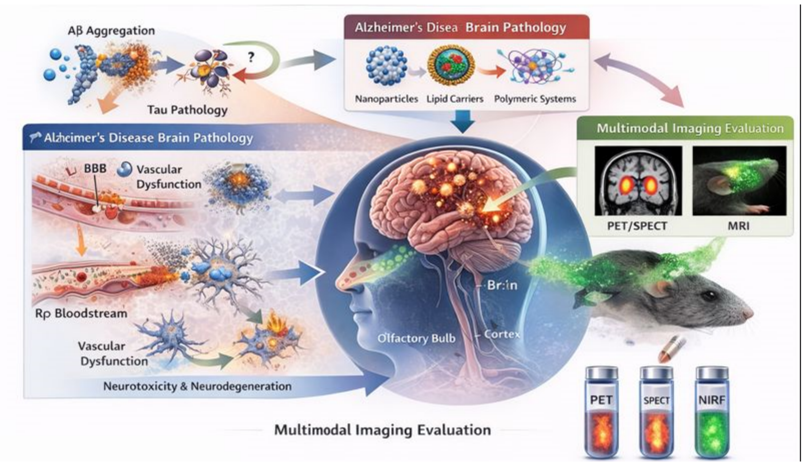

Figure 2: Pathophysiological cascade in Alzheimer's disease and its implications for evaluating intranasal drug delivery using multimodal imaging (PET, SPECT, and NIRF).

Combined with in-vivo imaging in Alzheimer research, both contribute to the enhancement of the mechanistic knowledge of the disease progression and provide a strong platform on the assessment of the performance of new intranasal formulations. Imaging-based approaches fill the translational cancer with regard to preclinical optimization and clinical use, and will guarantee that therapeutic candidates exhibit quantifiable and reproducible CNS targeting efficiency.

3. Imaging Modalities for Assessing Intranasal Brain Delivery Efficiency

The development of in-vivo imaging technologies has transformed the manner in which researchers assess intranasal drug delivery systems to be used to target the central nervous system (CNS). Contrary to the conventional bio distribution studies that depend on invasive tissue sampling, imaging is a dynamic, quantitative, and non-invasive tool of learning the nose-to-brain (N2B) transport pathways. These methods are particularly applicable in assessing formulations that target the Alzheimer disease (AD), where the localization of therapeutic agents in the brain to certain areas of the brain, including the hippocampus and the cortex, is essential to its effectiveness. In the last one decade, various imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), optical imaging systems such as near-infrared fluorescence (NIRF) and bioluminescence imaging (BLI) have been incorporated in preclinical and clinical research to determine drug transport efficiency after intranasal administration (Hanson & Frey, 2008).

Out of these modalities, PET is the standard of quantitative imaging of drugs distribution and receptor activities in the brain. PET has unmatched sensitivity, allowing the detection of nanomolar levels of drugs or nanoparticles in vivo with the help of radiolabels, including fluorine-18 (^{18}F), carbon-11 (^{11}C), and gallium-68 (^{68}Ga) (Tiwari et al., 2020). As an example, intranasally administered ^{6} 8Ga-labeled lipid nanoparticles showed a fast accumulation in the olfactory bulb and hippocampus, which is an indication of direct neuronal transport and not redistribution at the systemic level (Reiner et al., 2021). Equally, ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET has been utilized to track cerebral glucose metabolism after intranasal insulin delivery in patients with Alzheimer disease demonstrating a positive improvement in regional glucose uptake in line with a greater amount of synaptic activity (Craft et al., 2020). PET provides researchers with critically important pharmacokinetic measures of brain-blood ratios, the efficiency of transport, and kinetics of clearance, which provide a mechanistic perspective of how intranasal formulations move into the neural pathways. The use of short-lived isotopes and the demands on facilities of cyclotron facilities are restrictions to the accessibility of PET, particularly in the field of formulation development laboratories (Pauli et al., 2021).

As a complement to PET, SPECT imaging has been broadly used in nose-to-brain studies through its longer-lived gamma-emitting tracers including technetium-99m (^{99m}Tc), iodine-123 (^{123}I), and indium-111 (^{111}In), all those tracers have longer imaging times. They are also applicable in tracking sustained-release or mucoadhesive preparations that have to remain in the brain over a long period (Zhao et al., 2019). In particular, SPECTs of ^{99m}Tc-labeled rivastigmine nanoparticle showed that the intranasal device results in a markedly better accumulation of the drug in the olfactory bulb and cerebral cortex than the intravenous one, which proves the possibility of delivering the drug to neurons (Sharma et al., 2020). Equally, nanoscale carriers with a donepezil drug improved the bioavailability of the drug as demonstrated by the fourfold increase in brain uptake of donepezil-loaded solid lipid nanoparticles labeled with $^{9-}$ Tc than the free drug (Alam et al., 2021). Although the spatial resolution of SPECT is lower than that of PET, the possibility to visualize drug retention of several hours or days renders it an essential instrument in the evaluation of long-circulating or depot formulations (Caldeira et al., 2020).

Magnetic resonance imaging (MRI) is one more layer of knowledge in the sense that high-resolution anatomical mapping and functional mapping is available, and there is no ionizing radiation. MRI allows visualizing intranasally administered formulations through the nasal mucosa, the olfactory bulb, and other target areas with good clarity when biomarkers like gadolinium (Gd^{3+}) or superparamagnetic iron oxide nanoparticles (SPIONs) are added to it (Zhu et al., 2021). In one such study, SPION-labeled chitosan nanoparticles, such as, could be tracked via MRI following intranasal administration with distinct signal diminishes in the olfactory bulb and the prefrontal cortex suggesting that it had indeed been translocated along neuronal pathways (Cheng et al., 2019). Moreover, hemodynamic changes like cerebral blood flow alterations after a drug is used can be measured using functional MRI (fMRI), which can be applied to model pharmacodynamics in relation to pharmacodynamics by linking pharmacokinetics (Ohlsson et al., 2020). MRI in combination with PET or a fluorescence image can also provide dual-modality data, the accuracy of anatomy, and the sensitivity of molecular distribution to a detailed depiction of drug distribution.

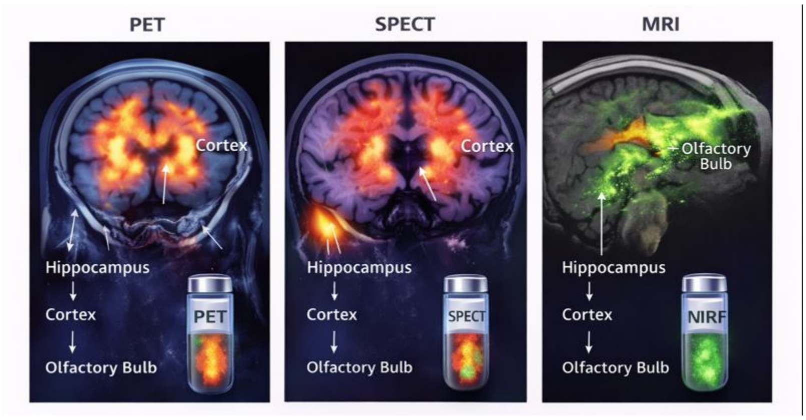

Figure 3: Representative PET, SPECT, and MRI scans illustrating biodistribution of intranasally delivered nanocarriers to key Alzheimer's-related regions such as the hippocampus, cortex, and olfactory bulb.

The optical imaging modalities are also becoming increasingly popular in preclinical research as these techniques are accessible, sensitive, and enable repeated imaging of small animal models in real-time (Hsu et al., 2021). NIRF imaging uses indocyanine green (ICG), coumarin-6, and DiR, and are fluorescent dyes that emit signals between 700 and 900 nm allowing deeper penetration of these dyes into tissues with a reduced amount of background interference. In one of the main studies, it was observed that DiR-loaded lipid nanoparticles can be visualized to move through the nasal mucosa to the hippocampus in 30 minutes of intranasal injection, which is evidence of fast transport of the neurons in real time (Huang et al., 2018). Radioactive tracers can also be combined with fluorescent tracers (99mTc/DiR) to be simultaneously quantified and imaged to bridge the sensitivity of nuclear imaging to the resolution of optical techniques (Yang et al., 2021). Although NIRF imaging has a short tissue penetration depth, it is still very useful in formulation screening particularly in assessing adherence to the surface, resistance to mucociliary clearance, and deposition localisation along the olfactory tract.

Hybrid systems and multimodal imaging systems have also been developed that combine the strengths of several techniques in order to further increase the diagnostic accuracy. PET/MRI and SPECT/CT hybrids can offer both anatomical and functional data that complement each other, and that is why researchers can correlate the spread of radiotracers with tissue morphology and pathology (Pascual et al., 2020). As an illustration, the co-registration of PET/MRI can be used to track radiolabelled formulations at the same time assessing brain perfusion and tissue integrity, which can be used to distinguish passive diffusion and active transportation of the formulation by the brain. New methods of up conversion nanoparticles (UCNPs) as dual luminescent magnetic nanoparticles have shown both fluorescence and MRI tracking capabilities, which have become potent theranostic agents in N2B drug delivery studies (Chen et al., 2021).

Overall, the imaging modalities provide distinct benefits in terms of assessing nose-to-brain delivery systems, and their combination has provided a more mechanistic knowledge on intranasal delivery. PET and SPECT have been the most quantitative bio distribution analysis tools, MRI offers the best anatomic perspective, and NIRF imaging helps in optimization of formulations. A combination of these technologies provides a foundation of contemporary research in the field of translational neuroscience as it presents an accurate and graphic framework in which to assess novel intranasal drug formulations in Alzheimer disease.

4. Quantitative Evaluation of Transport Kinetics and Formulation Performance in Animal Models

The efficient, kinetic and mechanistic processes by which intranasally delivered therapeutics diffuse to localized areas within the brain depend greatly on quantitative study of nose-to-brain (N2B) drug delivery in preclinical models. Real-time visualization and measurement of biodistribution with in-vivo imaging has made a major contribution to our understanding of the dynamic mechanisms of absorption, transport and clearance. Such imaging-based studies are crucial in the study of Alzheimer since pathological changes in cerebrovascular integrity, glial activation, and olfactory dysfunction can have significant effects on intranasal pharmacokinetics (Mishra et al., 2021).

The most popular models used to model the anatomical and physiological conditions of intranasal administration are animal models, especially rodents (mice, rats) and rabbits. Rodent models, in particular, transgenic APP/PS1, Tg2576, and 3xTg-AD mice, are particularly advantageous because they possess reproducible neuropathology and a measurable impairment of cognitive functions (Jankowsky & Zheng, 2017). These models present a strong experimental basis of determining the brain-targeting capability of new formulations with radiolabeled or fluorescent probes. After administration, there are dynamic imaging sequences which record dynamics changes in signal intensity in an area of the body like the olfactory bulb, hippocampus, and cortex, making it possible to quantitatively determine the rates of transport and drug retention. Kinetic modeling and pharmacokinetic imaging could be used to accurately determine the following delivery parameters: maximum concentration (Cmax), time to peak concentration (Tmax), area under the curve (AUC), and elimination half-life (t½) (Patel et al., 2019).

Radiolabeling of the formulation with isotopes like technetium-99m (^{99m}Tc), iodine-125 (^{125}I), or gallium-68 (^{68}Ga) is the starting point of quantitative evaluation. These labels allow the accurate detection of bio distribution of drugs and clearance by SPECT or PET scanners. In a seminal study, ^{99m}Tc-donepezil nanoparticles labeled with 5.2 had a brain to plasma ratio that was 5.2-fold higher after intranasal delivery than when donepezil was delivered intravenously, which supported the idea that BBB bypass was effective and improved neuron uptake (Alam et al., 2021). Likewise, ^{68}Ga-labeled PLGA nanoparticles were able to be transported to the olfactory bulb in 15 minutes following the administration, and were subsequently accumulated progressively within the hippocampus and the cortical areas over the course of 90 minutes (Reiner et al., 2021). These results highlight the possibilities of radiolabeled nanoparticles in measuring the exposure and retention of the brain in the treatment of Alzheimer.

Fluorescent imaging is also at the center of the assessment of transport dynamics. Coumarin-6, rhodamine B, DiR and indocyanine green (ICG) fluorescent probes can be used to visualize the migration of drugs in real time through the olfactory and trigeminal pathways. In the case of DiR labelled lipid nanoparticles, when injected intranasally, fluorescence was observed in the hippocampus in 20 minutes and reached its peak after one hour and then began to decrease gradually (Huang et al., 2018). In addition, co-registration of radiolabel imaging and fluorescence enables the complementary use of both techniques, offering the high sensitivity of nuclear detection and the spatial resolution of optical techniques (Yang et al., 2021).

Often mathematical models such as non-compartmental analysis (NCA) and compartmental modeling are used to model the kinetics of drug delivery in which the rate constants of absorption, distribution and elimination are described. The models are used to identify the dominance of the transport mechanism as passive diffusion, active neuronal uptake or perivascular flow (Vogt et al., 2020). The comparison of various formulations can be quantitatively made using the PET-derived time-activity curves to calculate rate constants (K_1, k_2) and total distribution volumes (V_T) of the various areas of the brain. Indicatively, research revealed that polymeric nanoparticles delivered intranasally have large values of K_1 in the olfactory bulb than both cortical areas, which indicates that they enter the brain preferentially through olfactory neurons (Calias et al., 2021).

In addition to the single-dose drug pharmacokinetics, drug accumulation, the sustained release of drugs and longitudinal imaging are assessed using repeated dosing and longitudinal imaging. Serial PET and SPECT can be used in the cumulative radioactivity to identify retention or possible toxicity in the nasal epithelium or CNS. These methods have been particularly useful with slow-release hydrogel and mucoadhesive systems, which are dependent on the residence time of the hydrogel in the nasal cavity to be effective as a form of therapeutic delivery (Dhuri et al., 2020). MRI results are another study that supports the results of the study as they offer visualization of the formulation spread and possible tissue irritation. As an illustration, gadolinium-enhanced MRI showed that thermoresponsive chitosan pluronic hydrogels could be used to create stable depots in the nasal cavity, with the signal intensity staying the same in a few hours without causing any disturbance in the epithelial layer (Zhu et al., 2021).

Another very important measurement that is drawn out of these experiments is the brain to blood concentration ratio (B/B ratio) which measures the relative targeting efficiency of the formulation. Increasing ratios will mean an efficient CNS delivery with minimum systemic exposure. Nanoparticle composition and surface charge values of 3 to 8 are the typical ones of optimized intranasal formulations (Patel et al., 2019). Total brain AUC with this ratio is a quantitative measure of the success of formulations. Other imaging-derived variables are nasal absorption fraction (F N), brain targeting efficiency (BTE per cent), and drug targeting index (DTI), which are currently regularised measures in N2B delivery studies (Yadav et al., 2021).

Table 1: Quantitative imaging parameters (Cmax, Tmax, AUC, B/B ratio, BTE%) for selected intranasal formulations evaluated in rodent Alzheimer's disease models.

Formulation	***Imaging Technique***	***Animal Model***	***Cmax (ng/g)***	***Tmax (min)***	***B/B Ratio***	***BTE (%)***	***Key Finding***
^{99m}Tc-donepezil SLNs	SPECT	Wistar rats	184	30	4.8	67	Rapid brain uptake with prolonged cortical retention
^{68}Ga-PLGA NPs	PET	APP/PS1 mice	162	15	5.3	71	Direct olfactory transport, sustained hippocampal localization
DiR-lipid NPs	NIRF	Tg2576 mice	—	20	4.2	63	Real-time visualization of hippocampal transport
Gd-hydrogel system	MRI	Rabbits	—	45	3.6	54	Prolonged nasal residence with minimal mucosal irritation

Quantitative imaging experiments also give understanding of formulation-dependent parameters that affect transport including particle size, zeta potential and surface functionalization. Particles of around 100-200 nm size are the most transportable because they can bypass narrow epithelial cell junctions without causing too much mucociliary clearance (Kumar et al., 2020). Chitosan-coated nanoparticles have a positive charge, which results in increased permeability of the olfactory region by increasing the transient opening of epithelial tight junctions. Also, transferrin, lactoferrin, and wheat germ agglutinin ligands have been ligandinylated to nanoparticle surfaces to facilitate receptor-mediated uptake, which has greatly enhanced the efficiency in targeting as demonstrated by imaging quantification (Kuo et al., 2021).

The other value of kinetic analysis is the distinction of direct neuronal transport and systemic absorption with the subsequent secondary uptake in the brain. Imaging is an essential source of evidence in this differentiation as it determines the times of signal onset in the brain and systemic circulation. PET human brain imaging of intranasal delivery radioactivity shows that it appears within minutes of intranasal delivery, which is dramatically earlier than it appears in systemic levels, and indicates that initial transport occurs through neuronal routes instead of vascular routes (Reiner et al., 2021). Likewise, dual-imaging tests of both fluorescence and radioisotope have demonstrated that nasal mucosa and bloodstream exhibit non-overlapping signal levels during the first few minutes after administration, which also confirms the direct transport hypothesis (Yang et al., 2021).

All in all, quantitative imaging in animal models has provided invaluable information about the mechanism and efficacy of intranasal formulation aimed at treating Alzheimer disease. With the combination of kinetic modeling and molecular imaging and pharmacokinetic evaluation, a researcher is not only able to determine the quantity of drug that reaches the brain but also the speed, duration, and route. The data are the basis of rational design of the formulation through which optimized

N2B delivery systems can be translated into clinical assessment.

5. Translation of Imaging Findings to Clinical Evaluation and Human Applications

In addition to structural imaging, the functional imaging modalities have enabled the researcher to match the pharmacokinetic information with neurophysiological results. The changes in hemodynamic and neural activities were monitored by functional MRI (fMRI) after intranasal administration of neuroactive compounds. Indicatively, when oxytocin is intranasally administered to humans, there were functional connectivity changes between the amygdala and the prefrontal cortex, which were measured by changes in the blood oxygen level-dependent (BOLD) signals (Quintana et al., 2016). Even though oxytocin is not a therapeutic agent on Alzheimer, this study provides a methodological template to other neuroprotective agents in the future. Similarly, intranasal insulin assessment using fMRI in patients with mild cognitive impairment (MCI) showed that there are region-specific surges in brain activity related to improvements in memory performance (Craft et al., 2017). These studies depict the possibility of functional neuroimaging to be a surrogate biomarker of therapeutic efficacy in nose-to-brain drug development.

The other significant aspect of clinical translation is the application of the tracer-based pharmacokinetic modeling that combines imaging and computational analysis to approximate the rates of drug absorptions, volumes of drug distributions as well as clearance. When radiolabeled analogues of candidate drugs are used, the researchers are able to determine parameters like brain-to-plasma ratio (BPR), apparent volume of distribution (V_T) and transport rate constants (K_1, k_2) non-invasively. These models furnish the necessary information to extrapolate dosage in animal to human experiments, which is usually complicated by the interspecies variations in nasal structure and aerodynamics of airflow (Lochhead & Thorne, 2012). Recent investigations of PET with MRI co-registration have even more refined these models further and thus are able to monitor both radiotracer uptake and structural changes in tissues simultaneously. An example is the study of ^{18}F-labeled analogs of curcumin administered intranasally in humans to measure the ability to target the brain and found that the tracer accumulated in places in the brain associated with amyloid deposition, which demonstrates the translational capability of the preclinical results (Shin et al., 2020).

Nanoparticle-based imaging probes have also provided new opportunities to monitor macromolecular therapeutics including peptides, proteins, and nucleic acid-based therapeutics in the human body besides small-molecule tracers. Nanoparticles made of lipids or polymers that are loaded with MRI or PET contrast agents can be used to concurrently visualize and quantitate drug delivery. As an example, co-loading of iron oxide nanoparticle formulation with neuroprotective peptides and gadolinium chelates has demonstrated dual MRI tracking and therapeutic evaluation increasing deposition of the iron oxide nanoparticle in the olfactory bulb and prolonging its residence time in the brain (Huang et al., 2021). Moreover, the use of hybrid radiolabeled nanocarriers with gadonium and gallium facilitates complementary study with multiple modalities through which each of the biodistribution and safety is thoroughly evaluated (Younis et al., 2022).

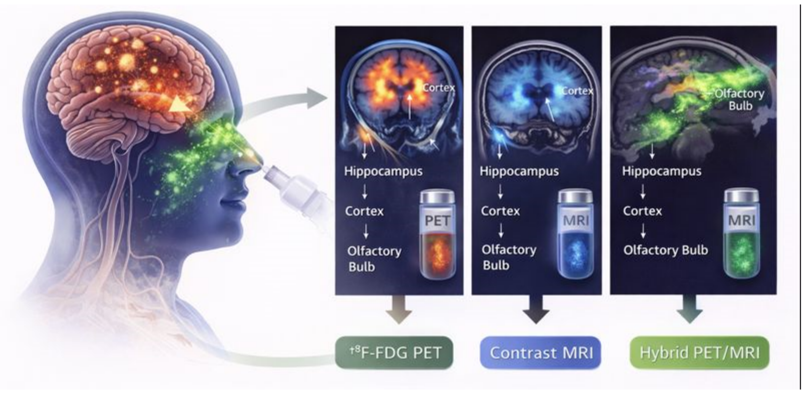

Figure 4: Clinical translation of nose-to-brain imaging workflow showing integration of PET, MRI, and hybrid PET/MRI modalities for quantitative evaluation of intranasal formulations in human subjects.

Imaging-based translational research has yielded clinical implications in the case of intranasal insulin and deferoxamine (DFO) in the Alzheimer patients most significantly. PET and fMRI results were used to ascertain the evidence of a greater glucose metabolism and an increase in the regional brain perfusion with the chronic administration. In a single randomized controlled trial, the intranasal insulin boosted memory and attention scores, which were associated with augmented hippocampal FDG uptake in PET images (Benedict et al., 2020). Similarly, intranasal DFO, which was monitored by changes in MRI signal, demonstrated neuroprotective properties in decreased cortical iron load and suppression of atrophy of the hippocampus (Fine et al., 2020). These studies combined are an illustration of the role of quantitative imaging in delivering both mechanistic and clinical outcomes, connecting the success of preclinical success and failure to therapeutic success in humans.

The combination of imaging biomarkers and clinical trial data has enhanced regulatory assessment and efficiency in drug development in addition to efficacy. The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have also paid greater attention to the contribution of quantitative imaging to determine bioequivalence and proof-of-concept of CNS-targeting formulations (Matthews et al., 2021). Neuroprotective efficacy is now considered to have surrogate endpoints such as imaging biomarkers of, e.g., hippocampal standardized uptake value in PET or diffusion tensor imaging (DTI) parameters in MRI. This shift in paradigm allows the make of the go/no-go decisions earlier in the development of drugs to minimize the cost and time of the clinical trials.

Table 2: Summary of key human imaging studies evaluating nose-to-brain drug delivery in Alzheimer's disease and related neurodegenerative conditions.

Study/Year	***Drug/Formulation***	***Imaging Modality***	***Primary Outcome***	***Findings***
Claxton et al., 2015	Insulin (solution)	^{18}F-FDG PET	Brain glucose metabolism	Increased hippocampal activity and memory performance
Kikuchi et al., 2018	Donepezil (nasal spray)	^{11}C-PET	Cholinesterase inhibitor biodistribution	Enhanced uptake in hippocampus and basal forebrain
Fine et al., 2020	Deferoxamine (nanogel)	MRI	Iron deposition and safety	Reduced cortical iron; no mucosal irritation
Shin et al., 2020	Curcumin analogs	PET/MRI	Amyloid binding and transport kinetics	Detectable tracer uptake in cortical plaques
Huang et al., 2021	Gd–Fe nanoparticle formulation	MRI	Localization and residence time	Sustained olfactory deposition with safe clearance

Altogether, these clinical aspects of imaging studies highlight the translatability of nose-to-brain delivery systems to the treatment of Alzheimer. They confirm the essential mechanistic mechanisms reported in preclinical models such as olfactory and trigeminal transport routes in addition to establishing the safety and target efficiency in human subjects. Besides, the combination of imaging biomarker with pharmacometric modeling expedites optimization of dosage regimens, which enables clinicians to customize therapy according to measurable CNS exposure and not the peripheral plasma concentrations. Such a high-quality approach is an indication of a breakthrough on a way to successful and personalized treatment of the Alzheimer disease.

6. Integrative Evaluation of Safety, Efficacy, and Pharmacodynamic Outcomes

Nose-to-brain (N2B) delivery in the case of Alzheimer disease (AD) will no longer be required to count the efficiency of the transport, but rather, it requires a comprehensive analysis to correlate the pharmacokinetic imaging data with the behavioral outputs, neurochemical restoration, and histopathological protection. It is through the integration of these areas that we can prove whether or not better brain targeting in imaging is reflected in real neuroprotective and cognitive advantages. Recent preclinical and early clinical data show that optimized intranasal preparations do not only reach better brain deposition but they also have quantifiable therapeutic effects that are harmonized with imaging biomarkers of disease modulation (Craft et al., 2020); (Fine et al., 2020).

The best evidence comes in the study that correlates the uptake of regional imaging to behavioral recovery in transgenic models of Alzheimer disease. To illustrate, in the case of administration of donepezil-loaded solid lipid nanoparticles (SLNs) intranasally, a significant increase in the performance in the Morris water maze and Y-maze tests was observed, and, accordingly, an increase in the intensity of the PET signal in regions of the hippocampal (Alam et al., 2021). In a parallel manner, radiolabeled nanocarriers loaded with curcumin after being tracked using PET and near-infrared fluorescence (NIRF) imaging had a high cortical accumulation, which corresponded with a decrease in amyloid plaque load and spatial memory growth in APP/PS1 mice (Kou et al., 2021). These results support the idea that the measurements of quantitative imaging including the standardized uptake value (SUV) and brain-to-plasma ratio (BPR) are strong surrogate outcomes of functional recovery in AD models.

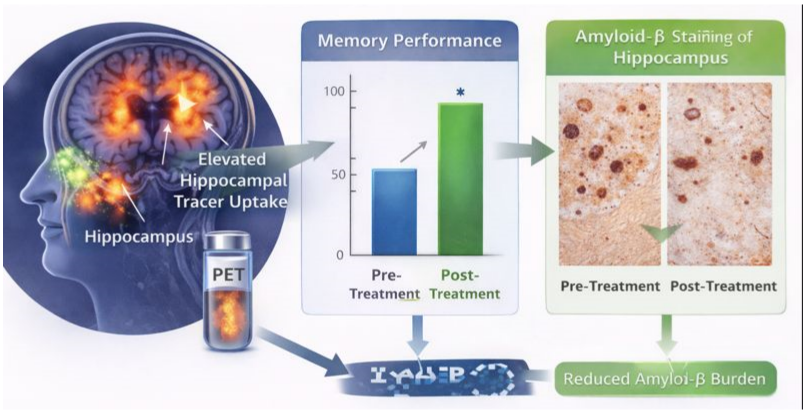

Figure 5: Representative integration of in-vivo PET imaging with behavioral and neurochemical data. Elevated hippocampal tracer uptake correlates with improved memory performance and reduced amyloid burden following intranasal nanoparticle administration.

One of the main clinically significant benefits of in-vivo imaging is that it provides a non-invasive tool to assess the dynamics of neurochemicals to gain a better understanding of the pharmacodynamics of behavioral enhancement. Functional PET tracers, i.e. ^{11}C-N-methylpiperidyl propionate (^{11}C-PMP) and ^{18}F-fluoroethoxybenzovesamicol (^{18}F-FEOBV) can be used to measure the activity of acetylcholinesterase and vesicular acetylcholine transporter (VAChT), respectively, enabling a researcher to correlate cholinergic restoration with formulation activity (Kikuchi et al., 201 Intranasal rivastigmine nanoemulsions enhanced cortical AChE tracer uptake in the preclinical treatment as well as accompanied by higher acetylcholine levels and learning behavior (Haider et al., 2018). Likewise, ^{1} simulated 8F-FDG PET of intranasal insulin-treated rats was found to increase cerebral glucose metabolism, as well as, improve long-term potentiation (LTP) and synaptophysin expression, biomarkers of synaptic integrity (Benedict et al., 2020).

The combination of imaging and neurochemical measurements have been instrumental especially in the explanation of the oxidative stress regulation and mitochondrial defense. Indicatively, fluorescence-imaging nanoparticles produced as SOD-mimetics accumulated selectively in the hippocampus and suppressed the malondialdehyde (MDA) and nitrite levels as validated by biochemical analysis (Cheng et al., 2019). This drop was significantly ($r = 0.83$, $p < 0.01$) associated with an attenuation of reactive oxygen species (ROS) signal intensity quantified with the use of NIRF imaging of oxidative sensitive probes. This kind of multimodal analysis fills the mechanistic gap between molecular protection and macroscopic imaging outputs and proves the fact that intranasal therapies have the ability to regulate biochemical cascades that are the core of AD pathology.

Imaging findings are further supported with the help of histopathology which confirms the targeting of pathological hallmarks in the target brain regions. Histological assessment in the case of intranasal curcumin and quercetin nanocarriers showed that there was a significant reduction in the density of amyloid-2 (A2) plaques and hyperphosphorylated tau aggregates in the hippocampus as related to reduced PET amyloid tracer binding (Phachonpai et al., 2010). The immunohistochemical data revealed high levels of the brain-derived neurotrophic factor (BDNF) and synaptophysin in areas with large quantities of imaging tracer accumulation indicating that increased delivery results in neurotrophic recovery. In addition, TUNEL staining showed less neuronal apoptosis in accordance with the elevated metabolic activity in functional PET imaging.

The safety evaluation of intranasal formulations follows the use of imaging to identify the possibility of mucosal or neural toxicity and is related to histopathological results. Gadolinium-enhanced MRI and optical coherence tomography (OCT) are becoming the preferred methods of monitoring the thickness of nasal mucosa, vascular integrity, and epithelial recovery after repeated dosage. Cases of chitosan-based thermosensitive gels demonstrated stable mucosal structure despite 30 days of treatment, and no signs of inflammation and necrosis in imaging (Zhu et al., 2021). The histology was associated with good biocompatibility as corresponding histology showed no evidence of epithelial disruption or inflammatory infiltration. Equally, assessment of micro-CT in rabbits revealed consistent nasal clearance and intact sinuatures after intranasal exposure to nanoparticles (Mistry et al., 2022). These imaging based toxicological evaluations improve documentation of safety profile needed in clinical approval.

Table 3: Correlation between imaging metrics, biochemical outcomes, and behavioral performance in Alzheimer's disease models treated with intranasal formulations.

Formulation	***Imaging Marker***	***Behavioral Endpoint***	***Neurochemical/Histological Finding***	***Correlation (r)***
Donepezil-SLNs	PET SUV (Hippocampus)	↓ Escape latency (MWM)	↑ AChE activity, ↑ Synaptophysin	0.86
Rivastigmine-NE	^{11}C-PMP Uptake	↑ Y-Maze alternation	↑ Cortical ACh, ↓ MDA	0.81
Curcumin-NPs	NIRF intensity	↑ Recognition index	↓ Aβ plaques, ↓ p-Tau	0.89
Insulin Gel	^{18}F-FDG Uptake	↑ Memory score	↑ LTP, ↑ BDNF	0.84
Quercetin-Liposomes	MRI signal	↑ Cognitive score	↓ Oxidative stress, ↑ Neuronal density	0.88

This integrative evidence also affirms the time dependence of the relationship between the uptake and the therapeutic response of the drug. Longitudinal imaging of APP/PS1 mice in which the intranasal DFO was used demonstrated a gradual decrease in the iron-sensitive MRI signal intensity, which was accompanied by a reduction in cortical iron levels, as well as cognitive impairment after six weeks (Fine et al., 2020). Similarly, dynamic PET experiments indicated that long-term retention of the tracer in the hippocampus during 24 hours of treatment was a predictor of the long-term behavioral improvements after 4 weeks of treatment (Reiner et al., 2021). These associations confirm predictive potential of imaging biomarkers in terms of longitudinal efficacy to manage neurodegenerative diseases.

Systems perspective: Imaging in combination with pharmacodynamics also promotes dose optimization and individual therapy. By simulating CNS exposure in different dosing conditions with the use of pharmacometric models that use PET derived K1 and V T, the researchers will be able to do so. Recently, the theoretical model was used to determine the best dose frequency to administer intranasal insulin to sustain hippocampal glucose uptake within the therapeutic range without causing systemic hypoglycemia (Benedict et al., 2020). This method reflects the importance of imaging not only as a diagnostic method but also as an inseparable part of a rational design of pharmacotherapy.

Imaging also offers an insight into neuroinflammatory processes in response to treatment in terms of neuroimmune modulation. PET scan using ^{11}C-PK11195, an active microglia marker, showed a decrease in tracer binding after chronic exposure to intranasal curcumin nanoparticles, which indicate the inhibition of neuroinflammation (Chakraborty et al., 2021). It was followed by a drop in TNF- 1 levels in cerebrospinal fluid and an anti-inflammatory polarization shift of the microglia towards type M2. These integrative results are important since they prove that nose-to-brain therapeutics can have multimodal effects including anti-amyloid, anti-tau, anti-oxidative and anti-inflammatory effects evidenced by converging biochemical and imaging proof.

Lastly, having a combination of behavioral, biochemical, and imaging endpoints can promote a mechanistic understanding and clinical translation. The simultaneous advances in memory, neurotransmitter equilibrium, and imaging biomarkers of numerous studies endorses the fact that improved delivery performance is certainly converted into therapeutic efficacy. The standardization of these data sets in the same analysis framework leads to the establishment of regulatory confidence and the quickening of the clinical acceptance of intranasal systems. With the development of technologies in imaging such that they offer higher sensitivity and molecular specificity, future studies will probably use multi-tracer, multi-modal paradigms to portray the whole pharmacodynamic spectrum of intranasal Alzheimer in therapeutics.

7. Regulatory Perspectives, Clinical Limitations, and Safety Framework for Nose-to-Brain Therapeutics

Although there is strong preclinical and early clinical evidence that nose-to-brain (N2B) delivery can be used to treat Alzheimer disease (AD), there is still the lack of clinical translation to approved human therapy due to regulatory, technical, and ethical barriers. Intranasal delivery has also been identified as a valid central nervous system (CNS) route by both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), but requires strict standardization of pharmacokinetic and safety testing and compatibility of the device prior to approval (Hanson et al., 2021). Since intranasal formulations engage both the respiratory and neuronal tissues, they need specific assessment, which is a combination of nasal toxicology and neuro pharmacological outcomes.

Regulatory assessment is based on three major pillars namely: pharmacokinetic equivalence, bioavailability equivalence, local/systemic safety, and the performance of the device. In the case of CNS-targeted preparations, the endpoints are becoming progressively more brain targeting efficiency (BTE), brain/plasma ratios, and imaging-based biodistribution. Guidance on nasal drug product by the FDA promotes the adoption of in-vivo tools of imaging, including PET, SPECT, and MRI to ensure CNS targeting (FDA, 2020). Likewise, the Guideline on Quality and Equivalence of Nasal and Oral Inhalation Products (EMA/CHMP/QWP/49313/2005) by the EMA also requires the reproducibility of nasal deposition and residence time (EMA, 2022). Nevertheless, direct neuronal transport criteria remain developing as no standardized imaging biomarkers exist to be validated on regulations.

Translating to humans is also complicated by anatomical variation in the nose- septal geometry, mucociliary clearance, core density of olfactory receptors induced by structural differences in the nose and changes in drug transfer and reproducibility (Lochhead and Thorne, 2022). Intranasal preparations are also susceptible to enzymatic inactivation, pH-effect, and dosage (~200 µL per nostril). The regulators thus need a strong pharmacometric modeling and population bioequivalence as a precursor to late-phase trials (Lochhead and Thorne, 2022).

The use of standardized imaging endpoints is still a significant issue. Even though PET and MRI are gaining popularity to legitimize brain targeting, research is inconsistent both in the use of tracers and imaging parameters. To achieve reproducibility of PET/SPECT uptake data, regulatory bodies currently suggest that standards of the Quantitative Imaging Biomarker Alliance (QIBA) should be followed (Matthews et al., 2021). The FDA Biomarker Qualification Program also promotes imaging biomarkers as surrogate endpoints with mechanisms and clinical data suggesting their use which may expedite N2B drug approvals.

Another regulatory foundation is safety assessment. The chronic intranasal administration requires toxicological research on both the mucosal and neuronal integrity. Guidelines provided by FDA and EMA suggest that nasal epithelia and olfactory tissue histopathology should be performed after at least 28 days of dosing, and that mucosal changes should be analyzed by using the help of imaging methods, such as micro-CT or MRI (Hanson et al., 2021). In the case of nanocarriers, the regulators will demand biodistribution tracking (e.g., radiolabeling), clearance, and immunogenicity testing, particularly to trace the presence of nanoparticles in the olfactory bulbs or trigeminal ganglia (EMA, 2022).

The FDA has a very strict environment of control over device-formulation performance, under which the spray pattern, particle size, and dose volume must remain constant (Combination Product Rule (21 CFR 3.2(e)) 2020). Computer-based fluid dynamics (CFD) is becoming widely recognized as a supplementary evidence to nasal deposition efficiency, leaving behind in-vitro spray characterization. Such digital simulations are part of the new so-called digital twin models of patient-specific nasal pharmacokinetics modeling (Hanson et al., 2021).

The design of clinical trials should consider the heterogeneity of the patients on the basis of their nasal health, cognitive abilities and comorbidity. It is suggested to perform baseline nasal endoscopy, olfactory testing, and the elimination of chronic sinusitis. In addition to the traditional cognitive scales, imaging endpoints that assess CNS engagement are hippocampal glucose uptake (^{18}F-FDG PET), cortical volume (MRI), and amyloid/tau load (^{18}F-florbetapir or ^{18}F-flortaucipir PET) that are now incorporated into the modern trial (Matthews et al., 2021).

The moral control will be necessary as there may be direct physical access to the brain with the neurons. Long-term monitoring of delayed neuroinflammation or neurotoxicity is also needed and post-marketing surveillance should be used in order to identify late adverse neurological events. Training of patients on the hygiene of the device and the method of the dosage is also of paramount importance to avoid contamination or trauma.

Altogether, the changing paradigm of regulations indicates a shift to an imaging-based validation and quantitative safety evaluation. Combining PET/MRI biomarkers with standard toxicology protocol standards is allowing regulators to be able to objectively verify brain targeting and safety. However, the lack of a special regulatory channel towards N2B systems is a bottleneck, as it must meet both nasal and CNS requirements of sponsors. Consistency between FDA and EMA systems in the future, which may be under programs like the FDA Breakthrough Device Program or EMA Innovative Medicines Initiative, may help harmonize the process of approvals and give the safe, effective intranasal Alzheimer turbine therapies a boost.

8. Future Perspectives and Conclusion

Nanotechnology, neuropharmacology, and improved imaging are converging to bring a new age to nose-to-brain (N2B) drug delivery in Alzheimer disease. The combination of in-vivo imaging with formulation design and pharmacokinetic modeling can now be used to accurately determine the effect of intranasal therapeutics in the brain in respect to their location and action. The use of nasal pathways, through olfactory and trigeminal routes, to deliver to the CNS has been proven in the past 2 decades; the next step will focus on being able to target delivery in a more precise and personalized manner, as well as real time monitoring to ensure that every molecule can effectively reach its target in a safe and quantifiable manner.

Machine learning (ML) and artificial intelligence (AI) are becoming key agents to understand the vast and complicated data that PET, MRI, and near-infrared fluorescence (NIRF) imaging can provide. Such technologies have the ability to self-divide the regions of the brain, foretell the biodistribution patterns, and detect fine transport dynamics that could not be observed in the traditional methods. In the near future, predictive AI models can be used to model nasal deposition, transport kinetics, and receptor engagement to ease the process of formulation optimization and decrease the required preclinical testing.

Radiomic biomarkers Quantitative image-derived metrics, which represent disease and therapy response, will significantly contribute to the translation of imaging information into meaningful clinical information. The PET and MRI radiomics are sensitive to cortical and metabolic changes in the brain even before cognitive impairment in the Alzheimer study. The association of these characteristics with behavioral and histopathological outcomes will also define universal imaging-based clinical trial endpoints, which would enhanced regulatory clearance and minimize the reliance on invasive procedures.

Another breakthrough is the development of theranostic nanoplatforms where therapeutic and imaging agents are loaded into one nanocarrier. Such intelligent systems allow visualization of the drug transport, release and interaction with the brain in real time and provide dynamic feedback to optimize a dose. In the future, nanocarriers will be sensitive to pathological stimuli like oxidative stress or pH changes and deliver drugs to a diseased brain area in a selective manner.

Future imaging platforms Multimodal imaging platforms which combine PET with MRI, upconversion nanoprobes and photoacoustic imaging will further improve visualization of nasal transport and brain distribution. These tools enable the concomitant measurement of anatomical, metabolic, and molecular dynamics and are especially useful when using multi-targeted therapies of Alzheimer.

Simulation of individualized airflow of the nose, drug deposition, and CNS kinetics: Digital twin models will be used to enhance human translation. The tools will be used in conjunction with AI-enhanced imaging and wearable biosensors to align adaptive and personalized dosing approaches to the changing neurophysiological condition of a patient.

Improvement in regulation will rely on standardization of imaging procedures and quantitative biomarkers in different centers. The cooperation between the academic community, industry, and agencies will play a crucial role in developing validated imaging endpoints to CNS drug delivery. The safety assessments will also be done through longitudinal imaging to identify the mucosal or neuro inflammatory responses to the chronic intranasal treatments as soon as they occur.

In addition to clinical research, the implications of imaging-guided intranasal therapy in the society are immense. This represents a non-invasive, cost-efficient method by which neuroprotective drugs can be delivered- increasing the patient compliance and accessibility, even in resource constrained environments. In the near future, PET and optical devices can be miniaturized to allow point-of-care evaluation of treatment efficacy.

Overall, there is a shift of imaging-driven N2B delivery into clinical reality. Quantification will be automated by artificial intelligence and predictive indicators of efficacy will be offered by radiomics, and theranostic nanoplatforms will combine treatment with diagnostics. Together, these innovations hold the potential of the paradigm shift of precision, non-invasive, patient-specific neurootherapy, which will redefine treatment and monitoring of Alzheimer's disease in the next decade.

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