TECHNOLOGY EVALUATION OF [^{11}C]PABA, A PROBE FOR BACTERIA INFECTION IMAGING

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Background:

Bacterial infections have large implications on the public health system. With the recent emergence of antibiotic resistance, novel bacterial diagnosis technologies are in dire needs. Current imaging techniques are heavily dependent on secondary inflammatory changes to localize disease such as, increased blood flow, and vascular permeability in computed tomography (CT) and magnetic resonance imaging (MRI). One of the major caveats with these studies is the reliance on indirect methods for detection of infection, thus limiting specificity. This is a major issue when imaging immunocompromised patients, as sensitivity is not as high, and prone to false negative results. These modalities are generally of limited value in detecting early disease regardless of the cause.

Antibiotics are commonly prescribed in cases of severe cases of bacterial infection, and inflammation. However, in cases such as sterile inflammation, it may cause harmful side effects. Moreover, antibiotic resistance is also one of the many concerns that can implicate major threats to the global health system. For this reason, novel diagnostic technologies that can distinguish between bacterial infection and sterile inflammation are needed to combat antibiotic resistance.

Technology:

Folate metabolic pathway is essential for cellular function in both prokaryotes and eukaryotes including DNA, and amino acid synthesis. Due to its crucial role in bacteria, folate biosynthesis has been one of the most compelling targets in antibiotic development. For example, anti-folate targets like dihydrofolate reductase (DHFR) have been utilized in antibiotics such as trimethoprim.

The inventors from the University of California, San Francisco have developed a molecular probe targeting bacteria-specific metabolic pathways using Positron Emission Tomography (PET) imaging (Mutch, 2018). They radiolabeled Para-aminobenzoic acid (PABA) with a radioisotope. ^{11}C to create a [^{11}C]PABA conjugate. The radiopharmaceutical uses PABA as a carrier for the radioactive isotope, and bacterial infection can be detected upon uptake of PABA by bacteria and visualized via PET imaging. PABA serves as a precursor for the biosynthesis of folic acid compounds in bacteria. The uptake and incorporation pathway of PABA is demonstrated in **Exhibit 1**. PABA is rapidly accumulated by a wide range of pathogenic bacteria, including metabolically quiescent bacteria and clinical strains. Mammals lack the enzymatic machinery to utilize PABA and rapidly clear it through urinary excretion. These qualities have made radiolabeled PABA as an ideal candidate for bacterial infection imaging.

The inventors claimed to have developed a novel synthetic method of production and purification of [^{11}C]PABA conjugate starting from Grignard precursors. Hydrochloric acid was used as a quencher a cyclotron system is required for the completion of this reaction. In the study, the inventor found [^{11}C]PABA has the potential to detect slowly dividing or “dormant” bacteria in vivo. In vitro data demonstrates [^{11}C]PABA was efficiently incorporated into both *Escherichia coli (E.coli) and Staphylococcus aureus (S. aureus)*.

Novelty, Utility, and Comparative Advantages:

In this section, the novelty, utility, and comparative advantages of [^{11}C]PABA will be discussed.

Novelty: PABA is a known compound that is approved by the U.S. Food and Drug Administration (FDA) for use as a sunscreen, and treatment for Peyronie's disease, a skin condition. Prior art searches found that radiolabeling of PABA is not a novel concept, and has already been performed in past literature, and claimed in a few patents, please refer to **Relevant Prior Art**. GE Healthcare Ltd published radiotracer compositions in the filed patent US20130209358A1 (**Relevant Prior Art, Patent 1)**. In the compositions, PABA was mentioned in their claims. It is very likely that their radiotracer compositions covered structures for [^{11}C]PABA. In past literature, PABA has been radiolabeled with a different isotope such as ^{3}H. [^{3}H]PABA is commercially available for laboratory purposes. For these reasons, the structure and composition of [^{11}C]PABA are not novel. Though, the inventors did claim that their method of synthesis is novel. **Exhibit 2** demonstrates the reaction mechanism for the radiosynthesis and purification of [^{11}C]PABA. Their method involves the use of Grignard precursor and quenched with hydrochloric acid. No similar method or reaction mechanism was found in the prior art search**.**

Utility: In the article, the inventors validated the broad sensitivity of [^{11}C]PABA for human pathogens through their in vitro and in vivo testing. More specifically, they demonstrated that [^{11}C]PABA was effective in treating *E. coli* and *S. aureus* strains. They also found that [^{11}C]PABA can differentiate active bacterial infection from sterile inflammation in mice. These findings highlight the utility of this invention. Though, due to the lack of human data, this invention is not asserted to have the utility of treatment for humans.

Competitive Advantages: Infections of normally sterile spaces can be difficult to sample, diagnose and treat. These can include musculoskeletal infections such as diabetic foot and hepatobiliary infections such as cholangitis. Biopsies are often performed to obtain samples from these sterile spaces for lab analysis. However, biopsies can be expensive, invasive, and time-consuming, and oftentimes, the region of interest is inaccessible. [^{11}C]PABA can be utilized as a cheap and non-invasive alternative to diagnose these infections for higher specificity. Although radiolabeling of PABA can be easily done using other isotopes, ^{11}C remains as an ideal isotope due to its short biological and physical half-life, and low radiation exposure. This short half-life property may also pose as a barrier to clinical application and will be discussed in the next section of the report.

Potential Concerns:

One major concern is related to the lack of intellectual property protection. As mentioned previously, PABA is already an established compound and has been radiolabeled in previous literature. Radiolabeling is an obvious technique and it has been widely used in commercial and pre-clinical settings. PABA can be radiolabeled using different isotopes and can still yield similar diagnostic potential. Although the utility is established, [^{11}C]PABA seems to lack novelty and inventive steps. Inability to fulfill these criteria means that this invention cannot be protected by patent law. Without patent protection, it will be difficult to hold market exclusivity rights for this invention, and prone to copyright infringement. There is also a possibility that PABA can be labeled to make probes for photoacoustic and fluorescence imaging. These imaging techniques are less invasive and may hold comparative advantages over [^{11}C]PABA in clinical practices.

Clinical application of [^{11}C]PABA is hindered because of the property of the isotope.^{11}C has a short half-life of 20.33 minutes, limiting the clinical use to facilities with an on-site cyclotron. In Canada, there are only 12 hospitals that house cyclotrons**.** Available statistic shows that there are only 45 publicly funded PET scanners in Canada. Geographical distribution of PET scanners and cyclotrons are shown by province in **Exhibit 3.** Prince Edward Island, Northwest Territories, Nunavut, and the Yukon do not have PET scanners, or cyclotron facilities; patients must be sent out to other provinces to receive the scan. These statistics show that clinical application in Canada may be limited to a certain amount of provinces. In contrast to Canada, the United States has abundant PET scanners and 150 cyclotron sites available for medical purposes. The geographical distribution of PET cyclotrons in the US is shown in **Exhibit 4.** Based on these results, Clinical application in the US is more favorable.

Competition is another major threat to this invention. Currently, there are few technologies available for bacteria imaging. For example, MolecuLight is an FDA-approved wound fluorescence imaging device that allows clinicians to quickly visualize bacteria and measure wounds at the point of care. Compared to [^{11}C PABA], fluorescence imaging is less invasive and does not require an expensive imaging scanner (Serena, 2019). MolecuLight is currently only effective in imaging external infections, though the company is hoping to improve its technology for internal infections in the future. This may pose as a threat in the future, as clinicians will most like choose MoleculeLight over[^{11}C]PABA for its non-invasive property. Moreover, there are various types of PET tracers for bacteria imaging mentioned in previous literature that has also shown promising results, with some currently undergoing clinical trials.

While other PET tracers in the literature show promising results, few have gotten to the clinical trial phase. One of the main reasons is the lack of standardized and reproducible infection models. Thus, the prediction of results in humans is difficult. In addition, very few studies considered the potential of bacteria mutations that may occur during an infection, thus changing their property. Mutations may alter the uptake of radiotracers and lead to inaccurate results.

Preliminary market analysis:

Bacterial infection is one the most common causes of sepsis. According to a report from Kalorama Information, the sepsis diagnostic market was valued at $990 million in 2019 and is expected to grow over the forecast period 2020-2024 as shown in **Exhibit 5**. This growth is fueled by factors such as the increased prevalence of sepsis and the emergence of antibiotic resistance. **Exhibit 6** shows that the laboratory testing segment held a significant revenue share in 2019 in the global sepsis diagnostic market. While Point-of-Care testing only accounted for less than 25% of the market share. [^{11}C]PABA would be falling under the laboratory testing segment as it requires a PET imaging system to get the result. North America held the dominant position in terms of revenue and is expected to hold its dominance over the forecast period of 2020-2024. This would be an ideal market target for [^{11}C]PABA due to the availability of cyclotrons, frequent product approvals, and partnership opportunities. Though, as mentioned previously, the availability of PET scanners and cyclotrons must also be considered to determine the most feasible market location.

Prospect for potential application and commercialization:

The antibiotic crisis is currently costing $1.4 billion annually and expected to cost up to $7.6 billion per year to the health care sector by 2050. There are no new antibiotic classes since 1983, and many are losing their effectiveness because of antibiotic resistance. For this reason, there is a need for diagnostic tools for bacterial infections to minimize unnecessary prescription of antibiotics. Sterile inflammation cases are tricky and most often misdiagnosed as bacterial infections. [^{11}C]PABA can effectively distinguish between bacterial infection and a sterile inflammation with high specificity. Therefore, clinical application is strong for this invention; however, it is only limited to facilities with onsite PET scanners and cyclotrons as mentioned previously. [^{11}C]PABA can be distributed as a reaction kit similar to other commercially available radiopharmaceuticals. The kit will include non-radioactive reagents and can be stored in hospital facilities for a long lifespan. The labeling reaction will be completed upon the addition of ^{11}C by a licensed cyclotron operator.

Due to a lack of intellectual property protection, [^{11}C]PABA is not a strong prospect for commercialization. As mentioned previously, the short half-life of ^{11}C can restrict supply to remote regions where the production of raw materials is negligible. Thus, [^{11}C]PABA can only be marketed to a limited number of market locations. Currently, oncology and cardiology hold a large share in the radiopharmaceutical market. As a result, it may be difficult to gather funding and support to commercialize the product. If the inventors can do follow-up studies to validate [^{11}C]PABA as an all-purpose radiopharmaceutical to image all bacterial strains, many more opportunities for commercialization and partnership will open up. For example, in Canada, the Centre for Probe Development and Commercialization (CPDC) and adMare Bioinnovative Radiopharmaceutical Initiative (CARI) will gather resources together to pave the road to the successful commercialization of radiopharmaceutical innovations. CARI offers support in many aspects of the development of the invention, such as regulatory affairs, business development, commercialization, IP strategies, chemistry manufacturing and controls support.

Conclusion:

In conclusion, the research for [^{11}C]PABA is still in the early phase, and further testing is needed to determine the full potential of this invention. Clinical application of [^{11}C]PABA is limited, as a result of ^{11}C's short half-life. ^{18}F is more clinically applicable, however, ^{18}F labeled PABA has been claimed in the US20190209103A1 patent application. Lack of novelty and obviousness are barriers that prevent [^{11}C]PABA from being granted patent protection, ultimately making it difficult for commercialization. As a potential investor, I would not invest in this product unless [^{11}C]PABA can be proven to work on all bacteria strains.

References:

Mutch CA, Ordonez AA, Qin H, et al. [^{11}C]Para-Aminobenzoic Acid: A Positron Emission Tomography Tracer Targeting Bacteria-Specific Metabolism. *ACS Infect Dis*. 2018;4(7):1067-1072. doi:10.1021/acsinfecdis.8b00061

Serena TE, Harrell K, Serena L, Yaakov RA. Real-time bacterial fluorescence imaging accurately identifies wounds with moderate-to-heavy bacterial burden. J Wound Care. 2019 Jun 2;28(6):346-357. doi: 10.12968/jowc.2019.28.6.346. PMID: 31166857.

Appendix:

Relevant Prior Art:

Patent

1. US20130209358A1: Radiotracer compositions
   Summary of claim: Methods for the preparation of radiopharmaceutical compositions. Includes methods of imaging the mammalian body using the radiopharmaceutical compositions
   Status: Abandoned in US and GB
2. US20190209103A1: Position imaging tomography imaging agent composition and method for bacterial infection
   Summary of claim: Using ^{19}F labeled fluorobenzoic (very similar structure to PABA) acid for imaging bacterial infection.
   Status: Pending in US
3. US20110217234A1: Imaging ligands
   Summary of claim: Labeled substrate selected from group consisting of p-aminobenzoic acid (PABA), 2,6-diaminopimelic acid (DAP), xylose (XYL), alpha-methyl-glucopyranoside (MGP), shikimic acid (SHIK), cellobiose (CB), mannitol (MAN) sorbitol (SOR) and derivatives thereof for use in monitoring infection
   Status: Pending in US, and EP
4. WO2020055951A1: Radiolabeled paba and derivatives thereof for use as functional renal imaging agents
   Summary of claim: The use of ^{11}C labeled PABA for functional renal imaging agents. A group of positron emitting radionuclide were also included in the claim as well.
   Status: Published in WO
5. US7691905B2: Inhibition of melanogenesis and melanoma metastasis with p-aminobenzoic acid (PABA)
   Summary of claim: Use of PABA to treat melanotic cancer.
   Status: Active in US JP, and DE Abandoned in CA, AU, and AT.
6. US10300156B2: Radiotracer compositions and methods
   Summary of claim: radiotracer compositions which comprises ^{18}F-labeld c-Met binding peptides for in vivo imaging using PABA as radioprotectant.
   Status: Active in CN, and US, Pending JP, and EP, Abandoned in GB

Literature:

(1) Ruiz-Bedoya CA, Ordonez AA, Werner RA, Plyku D, Klunk MH, Leal J, Lesniak WG, Holt DP, Dannals RF, Higuchi T, Rowe SP, Jain SK. ^{11}C-PABA as a PET Radiotracer for Functional Renal Imaging: Preclinical and First-in-Human Study. *J Nucl Med*. 2020 Nov;61(11):1665-1671. doi: 10.2967/jnumed.119.239806. Epub 2020 Mar 20. PMID: 32198314.
Relevant information: Use of ^{11}C-PABA as PET tracer for functional renal imaging

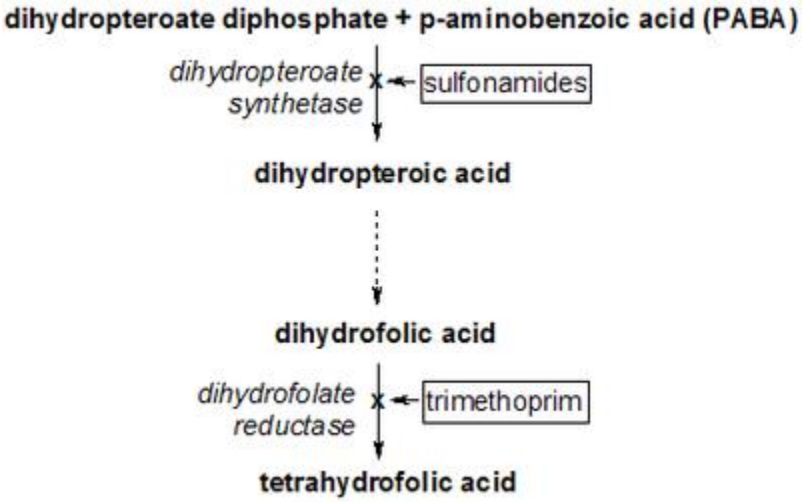
(2) Comley JC, Mullin RJ, Wolfe LA, Hanlon MH, Ferone R. A radiometric method for objectively screening large numbers of compounds against Pneumocystis carinii in vitro. *J Protozool*. 1991 Nov-Dec;38(6):144S-146S.
Relevant information: The use of ^{3}H labeled PABA for screening large numbers of compounds against Pneumocystis carnii in vitro.

(3) Kovacs JA, Powell F, Voeller D, Allegra CJ. Inhibition of Pneumocystis carinii dihydropteroate synthetase by para-acetamidobenzoic acid: possible mechanism of action of isoprinosine in human immunodeficiency virus infection. *Antimicrob Agents Chemother*. 1993 Jun;37(6):1227-31. doi: 10.1128/aac.37.6.1227. PMID: 7687120Relevant information: Used [^{3}H]PABA to validate incorporation of PABA when *P. carni* infected rats are treated with PAcBA, an inhibitor of PABA.

(4) Ordonez, et al. A systemic approach for developing bacteria-specific imaging tracers. *J Nucl Med*. 2017; 58:144-150. doi: 10.2967/jnumed.116.181792.
Relevant information: Radiolabeling method of PABA using ^{14}C and ^{3}H and in vitro and in vivo validation were performed as well in this article

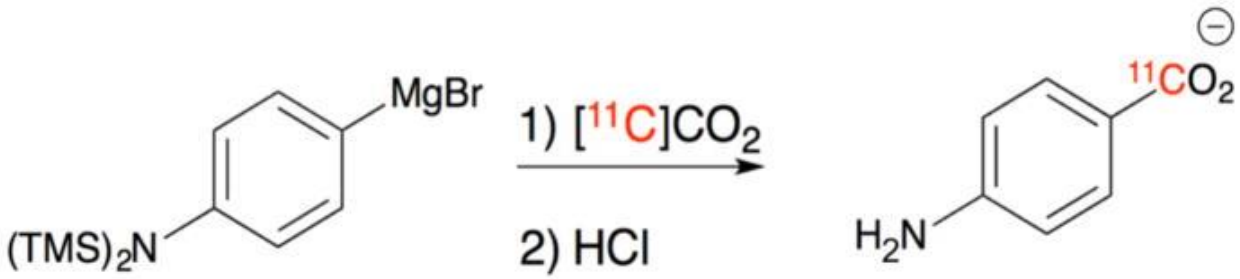
(5) Camilo et al. Folate Synthesized by Bacteria in the Human Upper Small Intestine Is Assimilated by the Host. *Gastroenterology*. 1996; 110(4), 991-998.
Relevant information: The use of ^{3}H labeled PABA as a precursor substrate for bacterial folate synthesis for in vitro validation.

Exhibit 1:



Source: Wikipedia

Exhibit 2:



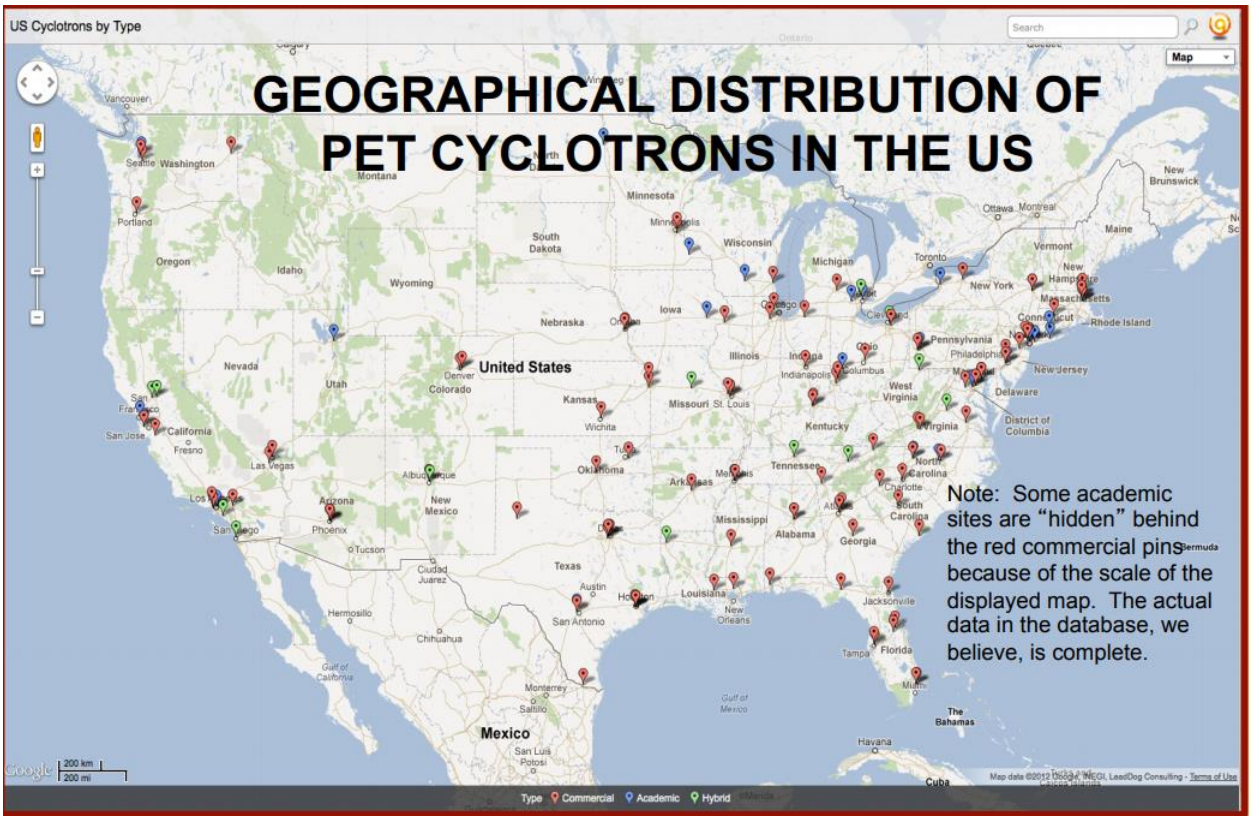
Source: Mutch et al, 2018

Exhibit 3: Geographical distribution of publicly funded PET scanners and cyclotron in Canada as of 2015

Province	Number of PET Scanners	Number of Cyclotrons
Ontario	16	5
Alberta	4	1
British Columbia	2	2
Saskatchewan	1	0
Newfoundland and Labrador	1	1
Manitoba	1	1
New Brunswick	2	FDG supplied by 1 site
Nova Scotia	1	1
Quebec	18	1
Prince Edward Island, Northwest Territories, Nunavut and Yukon.	No PET scanner and cyclotron are available at these provinces and territories as of 2015.	

Source: Canadian Agency for Drugs and Technologies in Health, 2015

Exhibit 4:



Source: J Nucl Med, 2012

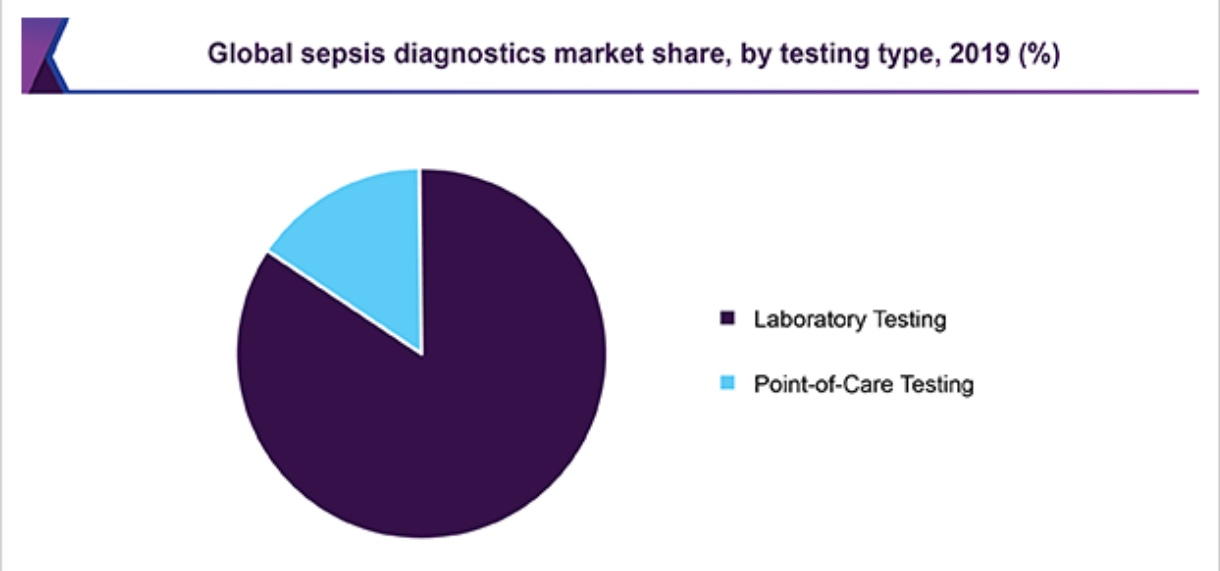
Exhibit 5:

Table 3-9: Global Molecular HAIs/Sepsis Testing Markets, by Region (2019-2024) ($, Million) (North America, Europe, APAC, RoW, Total)

	2019	2024	CAGR, %
North America	420	570	6.3
Europe	380	510	6.1
APAC	140	220	9.5
RoW	50	70	7.0
Total	990	1,370	6.7

Source: Kalorama Information, 2019

Exhibit 6:



Source: Grand View Research, 2019